BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-063524
Article Type:	Protocol
Date Submitted by the Author:	04-Apr-2022
Complete List of Authors:	Leenders, Nicoline; Vrije Universiteit Amsterdam, Department of Nephrology, Amsterdam Cardiovascular Sciences Douma, Caroline; Spaarne Gasthuis Hoofddorp, Department of Nephrology Hoenderop, Joost; Radboudumc, Department of Physiology, Radboud Institute for Molecular Life Sciences Vervloet, Marc; Amsterdam UMC, Vrije Universiteit Amsterdam, Department of Nephrology, Amsterdam Cardiovascular Sciences
Keywords:	Dialysis < NEPHROLOGY, NEPHROLOGY, Nephrology < INTERNAL MEDICINE, End stage renal failure < NEPHROLOGY

Magnesium in chronic hemodialysis (MAGIC-HD): a study protocol for a randomized controlled trial to determine feasibility and safety of using increased dialysate magnesium concentrations to increase plasma magnesium concentrations in people treated with hemodialysis.

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Abbreviations

AE, adverse event; CKD, chronic kidney disease; CRP, C-reactive protein; ECG, electrocardiography; PAC, premature atrial complex; PWV, pulse wave velocity; PTH, parathyroid hormone; PVC, premature ventricular complex; SOC, standard of care; SAE, serious adverse event; SPIRIT, Standard Protocol Items: Recommendation for Interventional Trials (SPIRIT)

Abstract

Introduction

People treated with hemodialysis are at increased risk for all-cause and cardiovascular mortality. Plasma magnesium concentration has been inversely associated with these risks. Therefore, plasma magnesium may be a new modifiable risk factor and an increase of dialysate magnesium concentration may be an easy, safe and effective way to increase plasma magnesium concentrations. Detailed information on modulating dialysate magnesium concentrations is limited in literature. Primary objective of this study is to determine the safety and feasibility to increase plasma magnesium concentrations in people treated with hemodialysis by means of sequentially increasing concentration of magnesium in the dialysate.

Methods and analysis

In this randomized double blinded standard of care controlled trial, fifty-three persons treated with hemodialysis, will be randomly allocated 2:1 to either a step-wise individually titrated increase of dialysate magnesium concentration from 0.50 to 0.75 to 1.00 mmol/L during 8 weeks, or a standard dialysate magnesium concentration of 0.50 mmol/L. Other study measurements include dietary records, questionnaires, electrocardiography (ECG), Holter registration and pulse wave velocity. The primary endpoint is pre-dialysis plasma magnesium after the long interdialytic interval at the end of week 8. In addition, the predictive effect of dialysate magnesium concentration and other baseline parameters and dialysis characteristics on plasma magnesium concentration, will be explored using linear mixed models. Safety endpoint is defined by the occurrence of hypermagnesemia above 1.25 mmol/L, or bradycardia or prolonged QTc interval detected on the ECG.

Ethics and dissemination

The study is conducted in accordance with the declaration of Helsinki as revised in 2013 and was approved by the Ethical Committee of the VU University Medical Center. The results of the study will be disseminated by publication in peer-reviewed scientific journals and presentation at national or international conferences in the field of interest.

Trial registration number

NTR 6568 / NL6393

Strengths and limitations

- Blinding and randomization prevents bias occurring from differences in life-style between groups, and enables objective collection and processing of data
- Dialysate concentrations will be individually titrated based on individual plasma magnesium concentrations by an independent physician
- Not only the effects of dialysate magnesium concentrations on plasma magnesium concentrations will be determined, but also the factors that are predictive for these effects
- Major limitation is that the study will not provide information on clinical outcomes including cardiovascular events and mortality, due to limited study duration

Introduction

People with chronic kidney disease (CKD) including those treated with dialysis are at increased risk for all-cause and cardiovascular mortality.[1] This increased risk persists after adjustment for traditional cardiovascular risk factors, indicating that other kidney specific factors contribute to the cardiovascular risk.[1] Recently, lower magnesium has been identified as a potential novel risk factor.[2] Magnesium is involved in many physiological functions, including energy metabolism and regulation of transmembrane transport of ions and consequently, it is essential for muscle function, cardiac rhythm and vascular tone.[3] In a meta-analysis of studies in people with CKD including those on dialysis, we showed that plasma magnesium concentration is inversely associated with all-cause and cardiovascular mortality, and that this not only applies for normal compared to low magnesium, but also revealed protective effects of magnesium above as compared to within the reference range (generally 0.70 – 1.05 mmol/L).[4] Magnesium concentration is also inversely associated with the risk for sudden death and with arrhythmia in people treated with hemodialysis.[5-7] In the general population, serum magnesium has been inversely associated with frequent or complex premature complexes, which predict prognosis including all-cause mortality in the general population.[8, 9] Moreover, serum magnesium was inversely associated with pulse wave velocity (PWV) in people treated with maintenance hemodialysis,[10] and in a short randomized cross-over study in people treated with hemodialysis, higher dialysate magnesium concentration compared to standard dialysate magnesium concentration decreased pulse wave velocity.[11] PWV is a marker of vascular stiffness and a strong predictor of cardiovascular outcome in people with CKD stage 4-5D.[12] In most studies that included multiple categories of plasma magnesium concentration in people with CKD including those treated with dialysis, there was a monotonic inverse association between magnesium and all-cause mortality.[4] We previously showed that a commonly used dialysate magnesium concentration of 0.50 mmol/L in hemodialysis, usually induces a decline of magnesium towards magnesium concentrations in the lower range of normal.[13] Therefore, an increase of dialysate magnesium concentration, may be an easy, safe and effective way to increase plasma magnesium concentrations in people treated with hemodialysis, without the need of oral supplementation. The results of one observational study suggest that there may be an optimal concentration of plasma magnesium in-between 1.15-1.27 mmol/L, with an increasing risk for mortality if magnesium values exceed this range.[14] Although these

findings were not confirmed by other studies that included magnesium values in this high range, this requires to take into account safety when increasing plasma magnesium concentrations.[6, 15, 16] In a previous 4-weeks trial by Bressendorf et al., increasing dialysate magnesium concentration from 0.50 to 1.00 mmol/L resulted in a 0.4 mmol/L increase of plasma magnesium concentration (95%-CI 0.3-0.5).[17] Here, we describe a randomized standard-of-care controlled trial of step-wise increment of dialysate magnesium concentration in people treated with hemodialysis. Primary objective is to determine the feasibility to increase plasma magnesium concentrations in individuals treated with hemodialysis by means of sequentially increasing concentration of magnesium in the dialysate. Secondary objectives are to determine safety of using higher dialysate magnesium concentrations, the effect of dialysate magnesium on plasma magnesium concentration, and to define which parameters are predictive for the increment of plasma magnesium concentration by increasing dialysate magnesium. We will also explore the effects of using higher dialysate magnesium on cardiac rhythm and pulse wave velocity.

Methods

Trial design

In this randomized, double-blind, standard of care (SOC) controlled multi-center trial, individuals treated with hemodialysis will be randomly allocated to either a stepwise increase of dialysate magnesium concentration from 0.50 to 1.00 mmol/L, or continue on a standard dialysate magnesium concentration of 0.50 mmol/L. The protocol was written in accordance with the Standard Protocol Items: Recommendation for Interventional Trials (SPIRIT) and registered at www.trialregister.nl (registration number NTR 6568 / NL6393).[18] The study is conducted in accordance with the declaration of Helsinki as revised in 2013 and was approved by the Ethical Committee of the VU University Medical Center (registration number 2017.408, NL62679.029.17).

Characteristics of participants and recruitment

Adult persons treated with hemodialysis on a 3-times weekly dialysis schedule, will be enrolled in the study. The in- and exclusion criteria are listed in table 1. Participants will be recruited from multiple centers in the Netherlands, including Amsterdam University Medical

Center location VU Medical Center, Amsterdam; Diapriva dialysis center, Amsterdam; Niercentrum aan de Amstel, Amstelveen; and Spaarne Gasthuis, Hoofddorp. Participants need to provide written informed consent prior to enrollment.

Table 1. Inclusion and exclusion criteria

Inclusion criteria
• Age ≥18 years • Hemodialysis with regular three times weekly dialysis schedule • Hemodialysis since at least 3 months • Standard dialysate magnesium concentration 0.50 mmol/L • Providing informed consent • Pre-dialysis plasma magnesium concentration ≤ 1.00 mmol/L after the long intra-dialytic interval
Exclusion criteria
• Intravenous magnesium supplementation (including total parenteral nutrition) in the last 2 weeks • Expected cessation of dialysis treatment within three months after inclusion or expected permanent or temporary dialysis center switch to a center not participating in the trial within three months after inclusion. • Prolongation of QTc interval: male >450 ms or female >460 ms on baseline ECG • Bradycardia: heart rate below 60 beats per minute on baseline ECG • Chronic arrythmia or cardiac conduction disorder other than atrial fibrillation or ventricular extrasystole that poses the patient at risk at the discretion of the treating physician. • Change of proton pump inhibitor prescription in the last 2 weeks

In order to be eligible to participate in this study, a subject must meet all of the inclusion criteria. A potential subject who meets any of the exclusion criteria will be excluded from participation in this study.

Intervention

In the intervention group, dialysate magnesium is increased stepwise, from 0.50 mmol/L at baseline, to 0.75 mmol/L during week 1-4, and to 1.00 mmol/L during week 5-8. The participant proceeds to the next increment step of dialysate magnesium concentration after week 4 only if pre-dialysis plasma magnesium after the long interdialytic interval is below 1.15 mmol/L in week 4. Otherwise, the dialysate magnesium concentration of 0.75 mmol/L is continued in week 5-8. After week 8, dialysate magnesium will be gradually reduced with 0.25 mmol/L in week 9 and thereafter return to the standard dialysate magnesium concentration of 0.50 mmol/L in week 10.

Participants in the control group are treated with a standard dialysate magnesium concentration of 0.50 mmol/L. (see Figure 1)

The dialysate contains a potassium concentration of 2 or 3 mmol/L, as determined in routine care by the treating physician based on individual needs. For the respective

magnesium concentrations, six dialysis concentrates are used in weeks 1-9 (Hemodialysis A-concentrate, D761, D987, D907, D283, D961 and D908, MTN Neubrandenburg GmbH, Neubrandenburg, Germany). In the composition of these concentrates, besides potassium based on individual needs, only the amount of magnesium chloride is different.

Study procedures and participant time line

The study procedures and participant time line are shown in Figure 2. After informed consent is provided by participants that meet in- and exclusion criteria, blood sampling and electrocardiography (ECG) are performed at baseline. Eligible persons meeting the criteria for plasma magnesium and ECG, as provided in table 1, are allocated to the either SOC or incremental magnesium dialysate. During the trial, blood sampling will be performed before and after the dialysis sessions following the long interdialytic interval weekly, and every dialysis session in week 1 and 5 to measure plasma magnesium concentration. In addition, in week 1, 5 and 9, blood is collected for measurements of potassium, bicarbonate, calcium, albumin, phosphate, parathyroid hormone (PTH), hemoglobin and C-reactive protein (CRP). For laboratory measurements, standard methods of the local laboratory are used. Participants record dietary intake for 3-days at baseline. From this record, dietary magnesium intake is extracted using the Dutch Food Composition Database (NEVO) by using the calculator on the website of the Dutch Nutrition Center.[19, 20] A questionnaire regarding the presence of subjective symptoms that can be associated with hypermagnesemia is completed at baseline, week 4 and 8. This is a 7-point yes or no questionnaire to ask if a participant experienced the following symptoms in the last week: nausea, vomiting, dizziness, drowsiness, reduced muscle strength, itching and leg cramps, and at baseline, also a question about chronic diarrhea and over-the-counter use of magnesium supplements is included. An electrocardiogram (ECG) is repeated before dialysis in week 4 and 8 to determine heart rhythm, frequency and QTc interval. In addition, at baseline and in week 8, participants undergo continuous heart rhythm monitoring and pulse wave velocity (PWV) measurements. Heart rhythm monitoring is performed using a Holter recorder (Fysiologic, Amsterdam, The Netherlands) for 48 hours including one dialysis sessions and one interdialytic period. Carotid-femoral PWV is measured using the Sphygmocor tonometer (Atcor Medical Pty Ltd, Software version 9.0, Sydney, Australia) preceding the midweek dialysis sessions. Participants are requested to avoid coffee, tea and smoking for 4 hours,

and alcohol for 12 hours preceding the measurements, as is recommended by the manufacturer of the device. The patient is placed in supine position, in a quiet environment at room temperature. After attachment of ECG electrodes and bedrest for at least 5 minutes, blood pressure is measured with an automated Omron device at least 3 times with a few minutes in-between, and until no substantial change occurs. Then, the last blood pressure measurement is recorded. The carotid to femoral artery distance is measured directly and multiplied by 0.8 to estimate the difference between cardiac-carotid and cardiac-femoral distance as recommended by expert consensus.[21] The pulse wave is recorded using the tonometer at the carotid and femoral site and then PWV is calculated automatically by the Sphygmocor software from entered blood pressure, distance and ECG and tonometer recordings. Measurements are performed at least twice or until two measurements are of appropriate quality. A measurement is considered of sufficient quality based on the criteria set by the manufacturer: adequate shape of detected signal of ECG and pulse wave, difference of heart rate ≤5 bpm between carotid and femoral measurement, ECG R-tops and pulse wave beginning are correctly identified by the software, and standard deviations of the ECG to carotid and of ECG to femoral time are both <6%. PWV measurement is not performed in participants with irregular heart rhythm, pacemaker rhythm, atrial fibrillation or flutter, heart frequency below 40 or above 160 bpm, 2nd or 3rd degree atrioventricular block, severe aortic valve stenosis or instable carotid plaque, as contra-indicated by the manufacturer. Furthermore, persons' characteristics are recorded at baseline and characteristics of the dialysis are recorded weekly for the time of dialysis after the long interdialytic interval and for every dialysis during the first and fifth week of intervention. Medication use and dosage is recorded at baseline and at week 8. During the trial, all participants receive three times weekly hemodialysis sessions according to their regular schedule. Changes of dialysis schedule during the study will be avoided as much as possible if there is no medical indication to change the scheme. Also, changes in prescription of proton pump inhibitors and magnesium-containing supplements, laxatives and phosphate binders will be avoided if clinically allowed.

Safety monitoring

If participants in the intervention group reach a plasma magnesium of 1.25 mmol/L or above, as noted by an unblinded independent nephrologist not involved in the trial (see

below), at any time point during follow-up in week 1 to 8, dialysate magnesium concentration will be reduced to the previous level in the next week. If plasma magnesium remains 1.25 mmol/L or above in the next week in intervention phase 2 (week 5-8), dialysate magnesium is further reduced one step (to 0.50 mmol/L). If a participant develops bradycardia with heart rate below 50 beats per minute (bpm), or prolonged QTc interval (>450 ms in male or >460 ms in female), noticed on electrocardiography (ECG) in week 4, dialysate magnesium concentration is also reduced to the previous step. (Figure 1) Plasma magnesium above 1.25 mmol/L, bradycardia < 50 bpm and prolonged QTc will be recorded as adverse event (AE). Serious adverse events (SAE's) will be recorded and reported to the primary investigator and medical ethical committee.

Main endpoints

Primary endpoint is pre-dialysis plasma magnesium concentration after the long interdialytic interval at the end of week 8, in the intervention group compared to the control group. The change of pre-dialysis plasma magnesium concentration after the long interdialytic interval from baseline to the end of week 8, will also be determined in the intervention group compared to the control group.

Secondary endpoints

Post-dialysis plasma magnesium concentrations after the dialysis sessions following the long interdialytic interval will also be determined. Safety endpoint is the safety of using higher magnesium concentrations in the dialysate, as indicated by the incidence of respectively hypermagnesemia (>1.25 mmol/L) at any timepoint, or bradycardia (defined as heart rate below 60 bpm) or prolonged duration of QTc interval (>450ms in male or >460ms in female) identified on the ECG in week 4 or 8. Other explorative endpoints include the change of PWV from baseline to week 8; and the number of complex premature ventricular complexes (complex PVC's), premature atrial complexes (PAC's), and heart rate variability as detected with Holter ECG monitoring. Complex PVC's are defined as PVC's that are multiform, repetitive or have a frequency of >30/h.[8] Furthermore, the following outcomes will be recorded: subjective symptoms that can be associated with hypermagnesemia determined from self-reporting in questionnaires in week 4 and 8; hospitalization; mortality; and cardiovascular events that lead to hospitalization or mortality including arrhythmia, cardiac

arrest, acute coronary syndrome, cerebrovascular accident, and hemorrhage from ruptured aneurysms of the abdominal aorta.

Blinding and randomization

Participants that fulfill all screening criteria, will be randomly allocated 2:1 in tranches of 6 to either the intervention group or the control group by the pharmacy according to a computer-generated random list. Participants, treating physicians, nurses and researchers are blinded to treatment allocation. Dialysate cannisters are re-labelled by the pharmacy for the individual participant per individual study week. Labels include information on participant name, date of birth, study week and dialysate potassium concentration, but no further information on dialysate composition. For the first study week, the pharmacy chooses the appropriate dialysate concentrate based on treatment allocation and the in routine individual care determined potassium concentration. From week 2 on, one independent nephrologist that is not blinded for treatment allocation, weekly decides upon the dialysate magnesium concentration, after review of plasma magnesium concentrations according to the algorithm shown in Figure 1. The pharmacy then re-labels the dialysate as prescribed by the independent nephrologist.

Sample size calculation

We previously performed a study to determine plasma magnesium concentrations and variability in people receiving 3-times weekly hemodialysis treatment with a standard dialysate magnesium concentration of 0.50 mmol/L.[13] That study showed a mean pre-dialysis plasma magnesium concentration of 0.88 ± 0.14 mmol/L.[13] After excluding pre-dialysis magnesium levels above 1.00 mmol/L from the analysis, mean pre-dialysis plasma magnesium level was 0.83 mmol/L in that study population. Based on these results, we expect a mean plasma magnesium concentration of 0.83 ± 0.14 mmol/L in the control group. Based on the results from the CONTRAST cohort analysis, in which plasma magnesium was associated with all-cause and cardiovascular mortality, we consider an increase of plasma magnesium concentration to 0.96 mmol/L in the intervention group relevant, which is a 0.13 mmol/L rise.[5] The required sample size calculated for two independent groups, based on the values just mentioned, a power of 0.80, probability of 0.05, and 2:1 randomization, would be 28 in the intervention and 14 in the control group. To

account for an estimated drop-out of 20%, the required sample size is 53 participants in total: 35 in the intervention and 18 in the control group.

Statistical analysis

Continuous variables will be expressed as mean and standard deviation (SD) for normally distributed variables or median and interquartile range (Q1-Q3) for non-parametric distributed variables. Categorical variables will be presented as number and percentage. The primary endpoint, pre-dialysis plasma magnesium after the long interdialytic interval at the end of week 8, will be compared between the intervention and control group, using univariable analysis with an unpaired Student's t-test if variables are normally distributed (if necessary after logarithmic transformation) and with Mann-Whitney U test if variables are non-normally distributed. The analysis is performed as intention to treat, including all participants that are still in study follow-up at week 8. The change of plasma magnesium from baseline to week 8, is first analyzed within each group using a paired Student's t-test, and then the difference from baseline to week 8 (delta) is compared between the groups using linear mixed models. The predictive effect of dialysate magnesium concentration and other baseline parameters and dialysis characteristics on plasma magnesium concentration, will be explored in two separate analyses (for pre-dialysis and post-dialysis concentration) using linear mixed models. In addition, we will explore which parameters are predictive for the increment of plasma magnesium concentration from baseline to week 8 after sequentially increasing dialysate magnesium concentration, using linear mixed models. For secondary endpoints, univariable analysis for within-group changes and between-group differences will be performed using respectively paired and unpaired Student's t-test or Mann-Whitney U tests for continuous variables, and chi-quadrate or Fisher's exact test for dichotomous variables. Linear mixed models will be used for multivariable analysis of secondary endpoints with repeated measures.

Data management

Data is recorded in electronic case report forms using the webbased software Castor EDC (Amsterdam, The Netherlands). All data is stored in coded form. The identification key is stored at the local study site only. Randomization codes are stored at the pharmacy. The randomization code will not be broken until follow-up of all participants is completed.

Dissemination

The results of this study will be offered for publication in international peer-reviewed journals. In addition, the results can be presented at national and international conferences and meetings in the field.

Patient and public involvement

This study protocol was reviewed and approved by the Dutch Kidney Foundation (DKF). A patient panel of the Dutch Renal Patients Association is involved in the review of research protocols submitted to the DKF. Investigators will communicate results to participants once the final results become available. The results will also be shared with patient organizations.

Discussion

This study aims to determine feasibility, safety, and predictive parameters for the effect of using dialysate with higher magnesium concentration to increase plasma magnesium concentrations in people treated with hemodialysis. In addition, this study will explore effects of using higher dialysate magnesium on cardiac rhythm and pulse wave velocity.

Relation with previous studies

In a previous study, we demonstrated that a commonly used dialysate magnesium concentration of 0.50 mmol/L generally induces a decline of plasma magnesium concentrations towards concentrations in the lower range of normal.[13] Detailed information in literature on the effects of increasing dialysate magnesium concentration on pre- and post-dialysis plasma magnesium concentrations and safety is sparse. Two other studies showed that a dialysate magnesium concentration of 0.75 mmol/L generally resulted in a relatively stable plasma magnesium concentration, with a mean pre-dialysis concentration of 1.2 mmol/L and mean post-dialysis concentrations of 1.1 up to 1.2 mmol/L.[22, 23] In another trial, a 4-weeks single-step increment of dialysate magnesium concentration from 0.50 to 1.00 mmol/L resulted in a 0.4 mmol/L increase (95%-CI 0.3-0.5) of pre-dialysis plasma magnesium concentration and a mean pre-dialysis plasma Mg concentration of 1.4 ± 0.2 mmol/L .[17] That study did not perform ECG nor Holter monitoring. As outlined in the

introduction, an observational study in people treated with hemodialysis found an inverse association between plasma magnesium concentrations and arrhythmia.[8] Moreover, in a short randomized cross-over study in people treated with hemodialysis, a dialysate magnesium concentration of 0.75 mmol/L compared to 0.50 mmol/L decreased pulse wave velocity.[11]

Strengths and limitations

The strengths of this study protocol are the blinding and randomization. Although the primary outcome is an objective outcome measure, blinding and randomization are essential to prevent bias occurring from changes in life styles including dietary magnesium intake. In addition, it is of relevance for objective collection and processing of data from questionnaires, pulse wave velocity measurements, electrocardiography, and reporting of SAE's. Another strength of this protocol is that dialysate magnesium concentration will be individually titrated based on individual plasma magnesium concentrations. In addition, not only the effect of dialysate magnesium will be determined, but also other factors that are predictive for this effect will be determined. The major limitation of this protocol, is that the study will not provide information on clinical outcomes including cardiovascular events and mortality, due to a limited duration of the study.

Potential impact

The study described in this protocol may provide relevant information on the effect of dialysate magnesium on plasma magnesium concentrations, the strategy for titration of dialysate magnesium based on individual needs, the safety of increasing plasma magnesium concentrations, and about factors that are predictive for the effect of dialysate magnesium on plasma magnesium concentration. This information may enable to safely increase plasma magnesium concentrations by individualized dialysate magnesium concentrations. In addition, this study will provide explorative data about the effects of increased dialysate magnesium concentration on intermediate cardiovascular outcomes including cardiac rhythm and PWV. The information provided by this study, may pave the way to larger long-term randomized controlled trials on the effects of increasing plasma magnesium concentrations on clinical outcomes including all-cause and cardiovascular mortality in people treated with hemodialysis. If plasma magnesium indeed improves clinically relevant

outcomes, and can be safely increased by means of individualized increasing dialysate magnesium concentrations, potentially large health benefits may be achieved if magnesium is increased slightly above the reference range by an increase of dialysate magnesium concentration. If so, the cost-effect ratio is likely low, as raising magnesium concentration is an inexpensive intervention. In addition, it would be an easy intervention that needs no additional patient effort and no oral supplementation would be needed in these persons that already experience a high pill burden. Therefore, the study described in this protocol may provide information of high relevance to patients, clinicians and health care providers and may eventually help to decrease morbidity and mortality in people treated with hemodialysis.

Safety considerations

Plasma magnesium concentrations are expected to rise up to values above the reference range by the increase of dialysate magnesium concentration. However, clinical symptoms of hypermagnesemia, typically are not observed before plasma magnesium concentrations exceed 2.0 mmol/L, which is high above the target concentrations in this study. Reported symptoms of hypermagnesemia (if plasma concentrations are above 2.0 mmol/L) include lethargy, drowsiness, flushing, nausea and vomiting , and diminished deep tendon reflexes.[24] In even more severe hypermagnesemia (plasma concentrations above 3.0 mmol/L) also somnolence, loss of deep tendon reflexes, hypotension and ECG changes can occur, and in extreme hypermagnesemia (above 5.0 mmol/L) complete heart block, cardiac arrest, apnea, paralysis and coma have been reported[24]. As a result of hemodialysis inherent techniques, the increment of (free) plasma magnesium is restricted by its concentration in the dialysate (maximally 1.00 mmol/L in this study), so overshoot to symptomatic concentrations is virtually impossible. The risk for severe or symptomatic hypermagnesemia is further minimized by intensive monitoring. As dialysate magnesium concentration is not further increased after a plasma magnesium concentration of 1.15 mmol/L is reached, and is reduced if plasma concentrations of 1.25 mmol/L are reached at any time point, severe or symptomatic hypermagnesemia will be prevented. These maximal target concentrations are set taking into account an observational study in people treated with hemodialysis that suggested an optimal magnesium concentration in-between 1.15-1.27 mmol/L and increased risk for mortality if magnesium exceeds 1.27 mmol/L.[14] Furthermore, for safety

reasons, individuals with bradycardia or a prolonged QTc interval on the ECG at baseline will be excluded from participation, and in individuals with bradycardia with a heart rate below 50 bpm or a prolonged QTc interval identified on the ECG in week 4, dialysate magnesium will be reduced. Based on these careful methods, the risk for individuals participating in this study is low. Considering the limited burden and risks associated with this study and a possible highly-relevant contribution to future improvement of treatment and prognosis in people treated with hemodialysis, the potential benefits outweigh the burden and possible risks.

Trial status

The trial is currently ongoing. The first participant was randomized on the 4th of April 2018 and up till now 43 of 53 participants have been randomized.

Funding

This work was supported by the Dutch Kidney Foundation (PhD grant no. 15OP02) and the PPP Allowance made available by Top Sector Life Sciences & Health to The Dutch Kidney Foundation to stimulate public-private partnerships (grant no. LSHM17034-HSGF).

Conflicts of interest statement

MV received research grants from Vifor, Amgen, Fresenius, and acted as consultant for Medice, Astra Zeneca, Vifor, Amgen, Fresenius, Otsuma, Kyowa Kirin. The other authors declare no conflicts of interest.

Authors contributions

NL, MV and JH conceived the study. NL and MV designed the study. NL wrote the manuscript and MV and JH revised the manuscript. Each author approved the final version of the manuscript.

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Figure legends

Figure 1. Flow chart of the study intervention. Numbers within frames represent dialysate magnesium concentrations in mmol/L. All other magnesium concentrations represent plasma magnesium concentrations, also in mmol/L. Mg, magnesium; ECG; electrocardiography; $^{\Delta}$, ECG criteria at baseline: bradycardia defined as heartrate <60 bpm or prolonged QTc interval >450ms in male or >460ms in female; $^{\Delta\Delta}$, ECG criteria in week 4: bradycardia with heart rate <50 bpm or prolonged QTc interval >450ms in male or >460ms in female; *, pre-dialysis plasma Mg concentration after the long interdialytic interval; **, plasma Mg concentration at any time point (pre-dialysis and post-dialysis measurements included).

Figure 2. Study procedures. dialysis 1, dialysis 2, dialysis 3: first, second, third dialysis session after the long interdialytic interval of the week respectively; pre: pre-dialysis; post: post-dialysis; blood sampling set 1: lithium heparin gel tube for plasma magnesium measurement; set 2: lithium heparin gel tube for plasma magnesium and potassium measurement; set 3: lithium heparin gel tube for plasma magnesium, potassium, calcium, albumin, phosphate, bicarbonate, CRP, and 2x EDTA tube for hemoglobin and PTH; set A: serum tube for biobanking in participants who provided additional informed consent; set B: 2x EDTA tube for biobanking in participants who provided additional informed consent; ECG: pre-dialysis electrocardiography; Questionnaire: 7-point yes or no questionnaire to ask if a participant experienced the following symptoms in the last week: nausea, vomiting, dizziness, drowsiness, reduced muscle strength, itching and leg cramps, in addition at baseline a question about chronic diarrhea and over-the-counter use of magnesium supplements is included; 3DDD: 3-days dietary diary including 1 dialysis weekday, 1 non-dialysis weekday, 1 non-dialysis weekend day; Holter: 48-hours electrocardiography registration including 1 dialysis session and 1 interdialytic interval; PWV: pulse wave velocity measurement before the mid-week dialysis session.

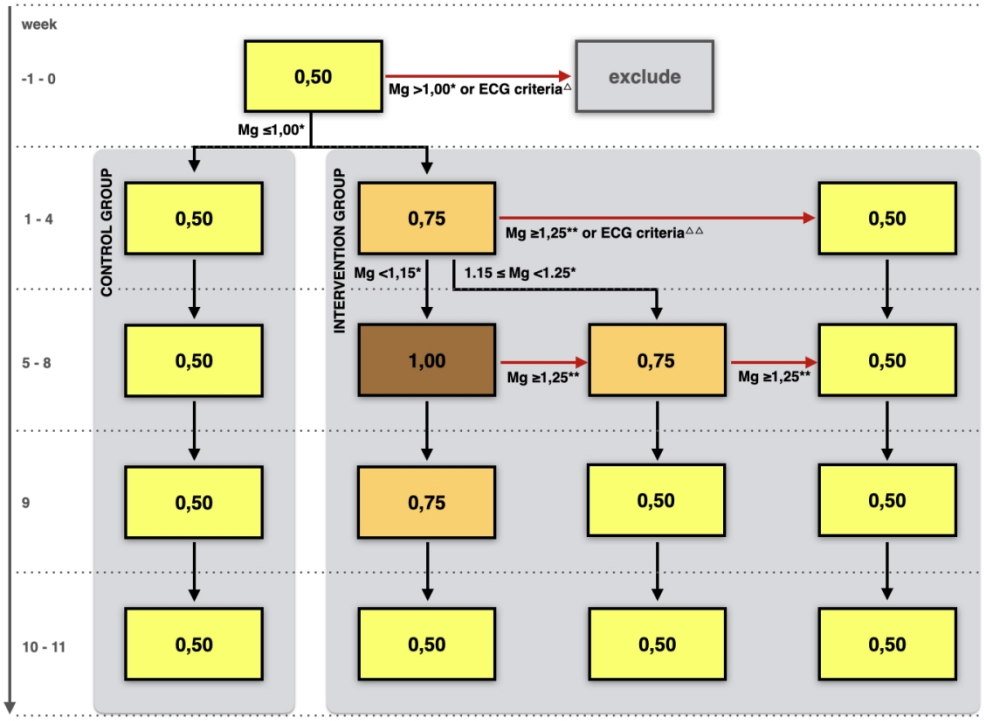

Figure 1. Flow chart of the study intervention. Numbers within frames represent dialysate magnesium concentrations in mmol/L. All other magnesium concentrations represent plasma magnesium concentrations, also in mmol/L. Mg, magnesium; ECG; electrocardiography; △, ECG criteria at baseline: bradycardia defined as heartrate <60 bpm or prolonged QTc interval >450ms in male or >460ms in female; △△, ECG criteria in week 4: bradycardia with heart rate <50 bpm or prolonged QTc interval >450ms in male or >460ms in female; *, pre-dialysis plasma Mg concentration after the long interdialytic interval; **, plasma Mg concentration at any time point (pre-dialysis and post-dialysis measurements included).

483x357mm (72 x 72 DPI)

Week	Dialysate	Blood sampling dialysis 1 pre	dialysis 1 post	dialysis 2 pre	dialysis 2 post	dialysis 3 pre	dialysis 3 post	ECG	Questionnaire	3DDD	Holter	PWV
-1	standard	set 1	set 1	set 1	set 1			X				
0	standard								X	X	X	X
1	study dialysate	set 3+A+B	set 2	set 1	set 1	set 1	set 1					
2	study dialysate	set 1	set 1									
3	study dialysate	set 1	set 1									
4	study dialysate	set 1	set 1					X	X			
5	study dialysate	set 3	set 2	set 1	set 1	set 1	set 1					
6	study dialysate	set 1	set 1									
7	study dialysate	set 1	set 1									
8	study dialysate	set 1	set 1					X	X		X	X
9	study dialysate	set 3+A	set 2									
10	standard	set 1	set 1									
11	standard	set 1	set 1									

Figure 2. Study procedures. dialysis 1, dialysis 2, dialysis 3: first, second, third dialysis session after the long interdialytic interval of the week respectively; pre: pre-dialysis; post: post-dialysis; blood sampling set 1: lithium heparin gel tube for plasma magnesium measurement; set 2: lithium heparin gel tube for plasma magnesium and potassium measurement; set 3: lithium heparin gel tube for plasma magnesium, potassium, calcium, albumin, phosphate, bicarbonate, CRP, and 2x EDTA tube for hemoglobin and PTH; set A: serum tube for biobanking in participants who provided additional informed consent; set B: 2x EDTA tube for biobanking in participants who provided additional informed consent; ECG: pre-dialysis electrocardiography; Questionnaire: 7-point yes or no questionnaire to ask if a participant experienced the following symptoms in the last week: nausea, vomiting, dizziness, drowsiness, reduced muscle strength, itching and leg cramps, in addition at baseline a question about chronic diarrhea and over-the-counter use of magnesium supplements is included; 3DDD: 3-days dietary diary including 1 dialysis weekday, 1 non-dialysis weekday, 1 non-dialysis weekend day; Holter: 48-hours electrocardiography registration including 1 dialysis session and 1 interdialytic interval; PWV: pulse wave velocity measurement before the mid-week dialysis session.

321x127mm (144 x 144 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Title page
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Abstract & 5
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	N/A
Funding	4	Sources and types of financial, material, and other support	15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Title page
	5b	Name and contact information for the trial sponsor	investigator initiated
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
	6b	Explanation for choice of comparators	6-7
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5, 10
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5-6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6-7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8-9

	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	N/A
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9-10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7-8
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10-11
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	5-6
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	10

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	10-11
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7-8, 11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	11
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11
Methods: Monitoring			

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	8-9
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	5
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N/A
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5-7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Figure 2
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	11

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	7-8, Figure 2

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Magnesium in chronic hemodialysis (MAGIC-HD): a study protocol for a randomized controlled trial to determine feasibility and safety of using increased dialysate magnesium concentrations to increase plasma magnesium concentrations in people treated with hemodialysis.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-063524.R1
Article Type:	Protocol
Date Submitted by the Author:	01-Aug-2022
Complete List of Authors:	Leenders, Nicoline; Vrije Universiteit Amsterdam, Department of Nephrology, Amsterdam Cardiovascular Sciences Douma, Caroline; Spaarne Gasthuis Hoofddorp, Department of Nephrology Hoenderop, Joost; Radboudumc, Department of Physiology, Radboud Institute for Molecular Life Sciences Vervloet, Marc; Amsterdam UMC location Vrije Universiteit Amsterdam, Department of Nephrology, Amsterdam Cardiovascular Sciences
Primary Subject Heading:	Renal medicine
Secondary Subject Heading:	Research methods
Keywords:	Dialysis < NEPHROLOGY, NEPHROLOGY, Nephrology < INTERNAL MEDICINE, End stage renal failure < NEPHROLOGY

Magnesium in chronic hemodialysis (MAGIC-HD): a study protocol for a randomized controlled trial to determine feasibility and safety of using increased dialysate magnesium concentrations to increase plasma magnesium concentrations in people treated with hemodialysis.

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Abbreviations

AE, adverse event; CKD, chronic kidney disease; CRP, C-reactive protein; ECG, electrocardiography; PAC, premature atrial complex; PWV, pulse wave velocity; PTH, parathyroid hormone; PVC, premature ventricular complex; SOC, standard of care; SAE, serious adverse event; SPIRIT, Standard Protocol Items: Recommendation for Interventional Trials (SPIRIT)

Abstract

Introduction

People treated with hemodialysis are at increased risk for all-cause and cardiovascular mortality. Plasma magnesium concentration has been inversely associated with these risks. Therefore, plasma magnesium may be a new modifiable risk factor and an increase of dialysate magnesium concentration may be an easy, safe and effective way to increase plasma magnesium concentrations. Detailed information on modulating dialysate magnesium concentrations is limited in literature. Primary objective of this study is to determine the safety and feasibility to increase plasma magnesium concentrations in people treated with hemodialysis by means of sequentially increasing concentration of magnesium in the dialysate.

Methods and analysis

In this randomized double blinded standard of care controlled trial, fifty-three persons treated with hemodialysis, will be randomly allocated 2:1 to either a step-wise individually titrated increase of dialysate magnesium concentration from 0.50 to 0.75 to 1.00 mmol/L during 8 weeks, or a standard dialysate magnesium concentration of 0.50 mmol/L. Other study measurements include dietary records, questionnaires, electrocardiography (ECG), Holter registration and pulse wave velocity. The primary endpoint is pre-dialysis plasma magnesium after the long interdialytic interval at the end of week 8. In addition, the predictive effect of dialysate magnesium concentration and other baseline parameters and dialysis characteristics on plasma magnesium concentration, will be explored using linear mixed models. Safety endpoint is defined by the occurrence of hypermagnesemia above 1.25 mmol/L, or bradycardia or prolonged QTc interval detected on the ECG.

Ethics and dissemination

The study is conducted in accordance with the declaration of Helsinki as revised in 2013 and was approved by the Ethical Committee of the VU University Medical Center. The results of the study will be disseminated by publication in peer-reviewed scientific journals and presentation at national or international conferences in the field of interest.

Trial registration number

NTR 6568 / NL6393

Strengths and limitations

- Blinding and randomization prevents bias occurring from differences in life-style between groups, and enables objective collection and processing of data
- Dialysate concentrations will be individually titrated based on individual plasma magnesium concentrations by an independent physician
- Not only the effects of dialysate magnesium concentrations on plasma magnesium concentrations will be determined, but also the factors that are predictive for these effects
- Major limitation is that the study will not provide information on clinical outcomes including cardiovascular events and mortality, due to limited study duration

Introduction

People with chronic kidney disease (CKD) including those treated with dialysis are at increased risk for all-cause and cardiovascular mortality.[1] This increased risk persists after adjustment for traditional cardiovascular risk factors, indicating that other kidney specific factors contribute to the cardiovascular risk.[1] Recently, lower magnesium has been identified as a potential novel risk factor.[2] Magnesium is involved in many physiological functions, including energy metabolism and regulation of transmembrane transport of ions and consequently, it is essential for muscle function, cardiac rhythm and vascular tone.[3] In a meta-analysis of studies in people with CKD including those on dialysis, we showed that plasma magnesium concentration is inversely associated with all-cause and cardiovascular mortality, and that this not only applies for normal compared to low magnesium, but also revealed protective effects of magnesium above as compared to within the reference range (generally 0.70 – 1.05 mmol/L).[4] Magnesium concentration is also inversely associated with the risk for sudden death and with arrhythmia in people treated with hemodialysis.[5-7] In the general population, serum magnesium has been inversely associated with frequent or complex premature complexes, which predict prognosis including all-cause mortality in the general population.[8, 9] Moreover, serum magnesium was inversely associated with pulse wave velocity (PWV) in people treated with maintenance hemodialysis,[10] and in a short randomized cross-over study in people treated with hemodialysis, higher dialysate magnesium concentration compared to standard dialysate magnesium concentration decreased pulse wave velocity.[11] PWV is a marker of vascular stiffness and a strong predictor of cardiovascular outcome in people with CKD stage 4-5D.[12] In most studies that included multiple categories of plasma magnesium concentration in people with CKD including those treated with dialysis, there was a monotonic inverse association between magnesium and all-cause mortality.[4] We previously showed that a commonly used dialysate magnesium concentration of 0.50 mmol/L in hemodialysis, usually induces a decline of magnesium towards magnesium concentrations in the lower range of normal.[13] Therefore, an increase of dialysate magnesium concentration, may be an easy, safe and effective way to increase plasma magnesium concentrations in people treated with hemodialysis, without the need of oral supplementation. The results of one observational study suggest that there may be an optimal concentration of plasma magnesium in-between 1.15-1.27 mmol/L, with an increasing risk for mortality if magnesium values exceed this range.[14] Although these

findings were not confirmed by other studies that included magnesium values in this high range, this requires to take into account safety when increasing plasma magnesium concentrations.[6, 15, 16] In a previous 4-weeks trial by Bressendorf et al., increasing dialysate magnesium concentration from 0.50 to 1.00 mmol/L resulted in a 0.4 mmol/L increase of plasma magnesium concentration (95%-CI 0.3-0.5).[17] Here, we describe a randomized standard-of-care controlled trial of step-wise increment of dialysate magnesium concentration in people treated with hemodialysis. Primary objective is to determine the feasibility to increase plasma magnesium concentrations in individuals treated with hemodialysis by means of sequentially increasing concentration of magnesium in the dialysate. Secondary objectives are to determine safety of using higher dialysate magnesium concentrations, the effect of dialysate magnesium on plasma magnesium concentration, and to define which parameters are predictive for the increment of plasma magnesium concentration by increasing dialysate magnesium. We will also explore the effects of using higher dialysate magnesium on cardiac rhythm and pulse wave velocity.

Methods

Trial design

In this randomized, double-blind, standard of care (SOC) controlled multi-center trial, individuals treated with hemodialysis will be randomly allocated to either a stepwise increase of dialysate magnesium concentration from 0.50 to 1.00 mmol/L, or continue on a standard dialysate magnesium concentration of 0.50 mmol/L. The protocol was written in accordance with the Standard Protocol Items: Recommendation for Interventional Trials (SPIRIT) and registered at www.trialregister.nl (registration number NTR 6568 / NL6393).[18] The study is conducted in accordance with the declaration of Helsinki as revised in 2013 and was approved by the Ethical Committee of the VU University Medical Center (registration number 2017.408, NL62679.029.17).

Characteristics of participants and recruitment

Adult persons treated with hemodialysis on a 3-times weekly dialysis schedule, will be enrolled in the study. The in- and exclusion criteria are listed in table 1. Participants will be recruited from multiple centers in the Netherlands, including Amsterdam University Medical

Center location VU Medical Center, Amsterdam; Diapriva dialysis center, Amsterdam; Niercentrum aan de Amstel, Amstelveen; and Spaarne Gasthuis, Hoofddorp. Participants need to provide written informed consent prior to enrollment.

Table 1. Inclusion and exclusion criteria

Inclusion criteria
• Age ≥18 years • Hemodialysis with regular three times weekly dialysis schedule • Hemodialysis since at least 3 months • Standard dialysate magnesium concentration 0.50 mmol/L • Providing informed consent • Pre-dialysis plasma magnesium concentration ≤ 1.00 mmol/L after the long intra-dialytic interval
Exclusion criteria
• Intravenous magnesium supplementation (including total parenteral nutrition) in the last 2 weeks • Expected cessation of dialysis treatment within three months after inclusion or expected permanent or temporary dialysis center switch to a center not participating in the trial within three months after inclusion. • Prolongation of QTc interval: male >450 ms or female >460 ms on baseline ECG • Bradycardia: heart rate below 60 beats per minute on baseline ECG • Chronic arrythmia or cardiac conduction disorder other than atrial fibrillation or ventricular extrasystole that poses the patient at risk at the discretion of the treating physician. • Change of proton pump inhibitor prescription in the last 2 weeks

In order to be eligible to participate in this study, a subject must meet all of the inclusion criteria. A potential subject who meets any of the exclusion criteria will be excluded from participation in this study.

Intervention

In the intervention group, dialysate magnesium is increased stepwise, from 0.50 mmol/L at baseline, to 0.75 mmol/L during week 1-4, and to 1.00 mmol/L during week 5-8. The participant proceeds to the next increment step of dialysate magnesium concentration after week 4 only if pre-dialysis plasma magnesium after the long interdialytic interval is below 1.15 mmol/L in week 4. Otherwise, the dialysate magnesium concentration of 0.75 mmol/L is continued in week 5-8. After week 8, dialysate magnesium will be gradually reduced with 0.25 mmol/L in week 9 and thereafter return to the standard dialysate magnesium concentration of 0.50 mmol/L in week 10.

Participants in the control group are treated with a standard dialysate magnesium concentration of 0.50 mmol/L. (see Figure 1)

The dialysate contains a potassium concentration of 2 or 3 mmol/L, as determined in routine care by the treating physician based on individual needs. For the respective

magnesium concentrations, six dialysis concentrates are used in weeks 1-9 (Hemodialysis A-concentrate, D761, D987, D907, D283, D961 and D908, MTN Neubrandenburg GmbH, Neubrandenburg, Germany). In the mineral composition of these concentrates, besides potassium based on individual needs, only the amount of magnesium chloride is different. Calcium concentration is these dialysates 1.25 mmol/L and the acidifier is acetate."

Study procedures and participant time line

The study procedures and participant time line are shown in Figure 2. After informed consent is provided by participants that meet in- and exclusion criteria, blood sampling and electrocardiography (ECG) are performed at baseline. Eligible persons meeting the criteria for plasma magnesium and ECG, as provided in table 1, are allocated to the either SOC or incremental magnesium dialysate. During the trial, blood sampling will be performed before and after the dialysis sessions following the long interdialytic interval weekly, and every dialysis session in week 1 and 5 to measure plasma magnesium concentration. In addition, in week 1, 5 and 9, blood is collected for measurements of potassium, bicarbonate, calcium, albumin, phosphate, parathyroid hormone (PTH), hemoglobin and C-reactive protein (CRP). For laboratory measurements, standard methods of the local laboratory are used. Participants record dietary intake for 3-days at baseline. From this record, dietary magnesium intake is extracted using the Dutch Food Composition Database (NEVO) by using the calculator on the website of the Dutch Nutrition Center.[19, 20] A questionnaire regarding the presence of subjective symptoms that can be associated with hypermagnesemia is completed at baseline, week 4 and 8. This is a 7-point yes or no questionnaire to ask if a participant experienced the following symptoms in the last week: nausea, vomiting, dizziness, drowsiness, reduced muscle strength, itching and leg cramps, and at baseline, also a question about chronic diarrhea and over-the-counter use of magnesium supplements is included. An electrocardiogram (ECG) is repeated before dialysis in week 4 and 8 to determine heart rhythm, frequency and QTc interval. In addition, at baseline and in week 8, participants undergo continuous heart rhythm monitoring and pulse wave velocity (PWV) measurements. Heart rhythm monitoring is performed using a Holter recorder (Fysiologic, Amsterdam, The Netherlands) for 48 hours including one dialysis sessions and one interdialytic period. Carotid-femoral PWV is measured using the Sphygmocor tonometer (Atcor Medical Pty Ltd, Software version 9.0, Sydney, Australia) preceding the midweek

dialysis sessions. Participants are requested to avoid coffee, tea and smoking for 4 hours, and alcohol for 12 hours preceding the measurements, as is recommended by the manufacturer of the device. The patient is placed in supine position, in a quiet environment at room temperature. After attachment of ECG electrodes and bedrest for at least 5 minutes, blood pressure is measured with an automated Omron device at least 3 times with a few minutes in-between, and until no substantial change occurs. Then, the last blood pressure measurement is recorded. The carotid to femoral artery distance is measured directly and multiplied by 0.8 to estimate the difference between cardiac-carotid and cardiac-femoral distance as recommended by expert consensus.[21] The pulse wave is recorded using the tonometer at the carotid and femoral site and then PWV is calculated automatically by the Sphygmocor software from entered blood pressure, distance and ECG and tonometer recordings. Measurements are performed at least twice or until two measurements are of appropriate quality. A measurement is considered of sufficient quality based on the criteria set by the manufacturer: adequate shape of detected signal of ECG and pulse wave, difference of heart rate ≤5 bpm between carotid and femoral measurement, ECG R-tops and pulse wave beginning are correctly identified by the software, and standard deviations of the ECG to carotid and of ECG to femoral time are both <6%. PWV measurement is not performed in participants with irregular heart rhythm, pacemaker rhythm, atrial fibrillation or flutter, heart frequency below 40 or above 160 bpm, 2nd or 3rd degree atrioventricular block, severe aortic valve stenosis or instable carotid plaque, as contra-indicated by the manufacturer. Furthermore, persons' characteristics are recorded at baseline and characteristics of the dialysis are recorded at baseline and weekly for the time of dialysis after the long interdialytic interval and for every dialysis during the first and fifth week of intervention. Recorded dialysis characteristics include modality (hemodialysis or hemodiafiltration), vascular access (catheter, fistula or graft), estimation of dialysis efficiency (Kt/Vurea per session according to Daugirdas' formula), treatment time per session, blood flow, dialysate flow and ultrafiltration volume. Medication use and dosage is recorded at baseline and at week 8. During the trial, all participants receive three times weekly hemodialysis sessions according to their regular schedule. Changes of dialysis schedule during the study will be avoided as much as possible if there is no medical indication to change the scheme. Also, changes in prescription of proton pump inhibitors

and magnesium-containing supplements, laxatives and phosphate binders will be avoided if clinically allowed.

Safety monitoring

If participants in the intervention group reach a plasma magnesium of 1.25 mmol/L or above, as noted by an unblinded independent nephrologist not involved in the trial (see below), at any time point during follow-up in week 1 to 8, dialysate magnesium concentration will be reduced to the previous level in the next week. If plasma magnesium remains 1.25 mmol/L or above in the next week in intervention phase 2 (week 5-8), dialysate magnesium is further reduced one step (to 0.50 mmol/L). If a participant develops bradycardia with heart rate below 50 beats per minute (bpm), or prolonged QTc interval (>450 ms in male or >460 ms in female), noticed on electrocardiography (ECG) in week 4, dialysate magnesium concentration is also reduced to the previous step. (Figure 1) Plasma magnesium above 1.25 mmol/L, bradycardia < 50 bpm and prolonged QTc will be recorded as adverse event (AE). Serious adverse events (SAE's) will be recorded and reported to the primary investigator and medical ethical committee.

Main endpoints

Primary endpoint is pre-dialysis plasma magnesium concentration after the long interdialytic interval at the end of week 8, in the intervention group compared to the control group. The change of pre-dialysis plasma magnesium concentration after the long interdialytic interval from baseline to the end of week 8, will also be determined in the intervention group compared to the control group.

Secondary endpoints

Post-dialysis plasma magnesium concentrations after the dialysis sessions following the long interdialytic interval will also be determined. Safety endpoint is the safety of using higher magnesium concentrations in the dialysate, as indicated by the incidence of respectively hypermagnesemia (>1.25 mmol/L) at any timepoint, or bradycardia (defined as heart rate below 60 bpm) or prolonged duration of QTc interval (>450ms in male or >460ms in female) identified on the ECG in week 4 or 8. Other explorative endpoints include the change of PWV from baseline to week 8; and the number of complex premature ventricular complexes

(complex PVC's), premature atrial complexes (PAC's), and heart rate variability as detected with Holter ECG monitoring. Complex PVC's are defined as PVC's that are multiform, repetitive or have a frequency of >30/h.[8] Furthermore, the following outcomes will be recorded: subjective symptoms that can be associated with hypermagnesemia determined from self-reporting in questionnaires in week 4 and 8; hospitalization; mortality; and cardiovascular events that lead to hospitalization or mortality including arrhythmia, cardiac arrest, acute coronary syndrome, cerebrovascular accident, and hemorrhage from ruptured aneurysms of the abdominal aorta.

Blinding and randomization

Participants that fulfill all screening criteria, will be randomly allocated 2:1 in tranches of 6 to either the intervention group or the control group by the pharmacy according to a computer-generated random list. Participants, treating physicians, nurses and researchers are blinded to treatment allocation. Dialysate cannisters are re-labelled by the pharmacy for the individual participant per individual study week. Labels include information on participant name, date of birth, study week and dialysate potassium concentration, but no further information on dialysate composition. For the first study week, the pharmacy chooses the appropriate dialysate concentrate based on treatment allocation and the in routine individual care determined potassium concentration. From week 2 on, one independent nephrologist that is not blinded for treatment allocation, weekly decides upon the dialysate magnesium concentration, after review of plasma magnesium concentrations according to the algorithm shown in Figure 1. The pharmacy then re-labels the dialysate as prescribed by the independent nephrologist.

Sample size calculation

We previously performed a study to determine plasma magnesium concentrations and variability in people receiving 3-times weekly hemodialysis treatment with a standard dialysate magnesium concentration of 0.50 mmol/L.[13] That study showed a mean pre-dialysis plasma magnesium concentration of 0.88 ± 0.14 mmol/L.[13] After excluding pre-dialysis magnesium levels above 1.00 mmol/L from the analysis, mean pre-dialysis plasma magnesium level was 0.83 mmol/L in that study population. Based on these results, we expect a mean plasma magnesium concentration of 0.83 ± 0.14 mmol/L in the control

group. Based on the results from the CONTRAST cohort analysis, in which plasma magnesium was associated with all-cause and cardiovascular mortality, we consider an increase of plasma magnesium concentration to 0.96 mmol/L in the intervention group relevant, which is a 0.13 mmol/L rise.[5] The required sample size calculated for two independent groups, based on the values just mentioned, a power of 0.80, probability of 0.05, and 2:1 randomization, would be 28 in the intervention and 14 in the control group. To account for an estimated drop-out of 20%, the required sample size is 53 participants in total: 35 in the intervention and 18 in the control group.

Statistical analysis

Continuous variables will be expressed as mean and standard deviation (SD) for normally distributed variables or median and interquartile range (Q1-Q3) for non-parametric distributed variables. Categorical variables will be presented as number and percentage. The primary endpoint, pre-dialysis plasma magnesium after the long interdialytic interval at the end of week 8, will be compared between the intervention and control group, using univariable analysis with an unpaired Student's t-test if variables are normally distributed (if necessary after logarithmic transformation) and with Mann-Whitney U test if variables are non-normally distributed. The analysis is performed as intention to treat, including all participants that are still in study follow-up at week 8. The change of plasma magnesium from baseline to week 8, is first analyzed within each group using a paired Student's t-test, and then the difference from baseline to week 8 (delta) is compared between the groups using linear mixed models. The predictive effect of dialysate magnesium concentration and other baseline parameters and dialysis characteristics on plasma magnesium concentration, will be explored in two separate analyses (for pre-dialysis and post-dialysis concentration) using linear mixed models. In addition, we will explore which parameters are predictive for the increment of plasma magnesium concentration from baseline to week 8 after sequentially increasing dialysate magnesium concentration, using linear mixed models. For secondary endpoints, univariable analysis for within-group changes and between-group differences will be performed using respectively paired and unpaired Student's t-test or Mann-Whitney U tests for continuous variables, and chi-quadrate or Fisher's exact test for dichotomous variables. Linear mixed models will be used for multivariable analysis of secondary endpoints with repeated measures.

Data management

Data is recorded in electronic case report forms using the webbased software Castor EDC (Amsterdam, The Netherlands). All data is stored in coded form. The identification key is stored at the local study site only. Randomization codes are stored at the pharmacy. The randomization code will not be broken until follow-up of all participants is completed.

Dissemination

The results of this study will be offered for publication in international peer-reviewed journals. In addition, the results can be presented at national and international conferences and meetings in the field.

Patient and public involvement

This study protocol was reviewed and approved by the Dutch Kidney Foundation (DKF). A patient panel of the Dutch Renal Patients Association is involved in the review of research protocols submitted to the DKF. Investigators will communicate results to participants once the final results become available. The results will also be shared with patient organizations.

Discussion

This study aims to determine feasibility, safety, and predictive parameters for the effect of using dialysate with higher magnesium concentration to increase plasma magnesium concentrations in people treated with hemodialysis. In addition, this study will explore effects of using higher dialysate magnesium on cardiac rhythm and pulse wave velocity.

Relation with previous studies

In a previous study, we demonstrated that a commonly used dialysate magnesium concentration of 0.50 mmol/L generally induces a decline of plasma magnesium concentrations towards concentrations in the lower range of normal.[13] Detailed information in literature on the effects of increasing dialysate magnesium concentration on pre- and post-dialysis plasma magnesium concentrations and safety is sparse. Two other studies showed that a dialysate magnesium concentration of 0.75 mmol/L generally resulted in a

relatively stable plasma magnesium concentration, with a mean pre-dialysis concentration of 1.2 mmol/L and mean post-dialysis concentrations of 1.1 up to 1.2 mmol/L.[22, 23] In another trial, a 4-weeks single-step increment of dialysate magnesium concentration from 0.50 to 1.00 mmol/L resulted in a 0.4 mmol/L increase (95%-CI 0.3-0.5) of pre-dialysis plasma magnesium concentration and a mean pre-dialysis plasma Mg concentration of 1.4 ± 0.2 mmol/L .[17] That study did not perform ECG nor Holter monitoring. As outlined in the introduction, an observational study in people treated with hemodialysis found an inverse association between plasma magnesium concentrations and arrhythmia.[7] Moreover, in a short randomized cross-over study in people treated with hemodialysis, a dialysate magnesium concentration of 0.75 mmol/L compared to 0.50 mmol/L decreased pulse wave velocity.[11]

Strengths and limitations

The strengths of this study protocol are the blinding and randomization. Although the primary outcome is an objective outcome measure, blinding and randomization are essential to prevent bias occurring from changes in life styles including dietary magnesium intake. In addition, it is of relevance for objective collection and processing of data from questionnaires, pulse wave velocity measurements, electrocardiography, and reporting of SAE's. Another strength of this protocol is that dialysate magnesium concentration will be individually titrated based on individual plasma magnesium concentrations. In addition, not only the effect of dialysate magnesium will be determined, but also other factors that are predictive for this effect will be determined. The major limitation of this protocol, is that the study will not provide information on clinical outcomes including cardiovascular events and mortality, due to a limited duration of the study.

Potential impact

The study described in this protocol may provide relevant information on the effect of dialysate magnesium on plasma magnesium concentrations, the strategy for titration of dialysate magnesium based on individual needs, the safety of increasing plasma magnesium concentrations, and about factors that are predictive for the effect of dialysate magnesium on plasma magnesium concentration. This information may enable to safely increase plasma magnesium concentrations by individualized dialysate magnesium concentrations. In

addition, this study will provide explorative data about the effects of increased dialysate magnesium concentration on intermediate cardiovascular outcomes including cardiac rhythm and PWV. The information provided by this study, may pave the way to larger long-term randomized controlled trials on the effects of increasing plasma magnesium concentrations on clinical outcomes including all-cause and cardiovascular mortality in people treated with hemodialysis. If plasma magnesium indeed improves clinically relevant outcomes, and can be safely increased by means of individualized increasing dialysate magnesium concentrations, potentially large health benefits may be achieved if magnesium is increased slightly above the reference range by an increase of dialysate magnesium concentration. If so, the cost-effect ratio is likely low, as raising magnesium concentration is an inexpensive intervention. In addition, it would be an easy intervention that needs no additional patient effort and no oral supplementation would be needed in these persons that already experience a high pill burden. Therefore, the study described in this protocol may provide information of high relevance to patients, clinicians and health care providers and may eventually help to decrease morbidity and mortality in people treated with hemodialysis.

Safety considerations

Plasma magnesium concentrations are expected to rise up to values above the reference range by the increase of dialysate magnesium concentration. However, clinical symptoms of hypermagnesemia, typically are not observed before plasma magnesium concentrations exceed 2.0 mmol/L, which is high above the target concentrations in this study. Reported symptoms of hypermagnesemia (if plasma concentrations are above 2.0 mmol/L) include lethargy, drowsiness, flushing, nausea and vomiting , and diminished deep tendon reflexes.[24] In even more severe hypermagnesemia (plasma concentrations above 3.0 mmol/L) also somnolence, loss of deep tendon reflexes, hypotension and ECG changes can occur, and in extreme hypermagnesemia (above 5.0 mmol/L) complete heart block, cardiac arrest, apnea, paralysis and coma have been reported[24]. As a result of hemodialysis inherent techniques, the increment of (free) plasma magnesium is restricted by its concentration in the dialysate (maximally 1.00 mmol/L in this study), so overshoot to symptomatic concentrations is virtually impossible. The risk for severe or symptomatic hypermagnesemia is further minimized by intensive monitoring. As dialysate magnesium concentration is not

further increased after a plasma magnesium concentration of 1.15 mmol/L is reached, and is reduced if plasma concentrations of 1.25 mmol/L are reached at any time point, severe or symptomatic hypermagnesemia will be prevented. These maximal target concentrations are set taking into account an observational study in people treated with hemodialysis that suggested an optimal magnesium concentration in-between 1.15-1.27 mmol/L and increased risk for mortality if magnesium exceeds 1.27 mmol/L.[14] Furthermore, for safety reasons, individuals with bradycardia or a prolonged QTc interval on the ECG at baseline will be excluded from participation, and in individuals with bradycardia with a heart rate below 50 bpm or a prolonged QTc interval identified on the ECG in week 4, dialysate magnesium will be reduced. Based on these careful methods, the risk for individuals participating in this study is low. Considering the limited burden and risks associated with this study and a possible highly-relevant contribution to future improvement of treatment and prognosis in people treated with hemodialysis, the potential benefits outweigh the burden and possible risks.

Trial status

The trial is currently ongoing. The first participant was randomized on the 4th of April 2018 and up till now 43 of 53 participants have been randomized.

Funding

This work was supported by the Dutch Kidney Foundation (PhD grant no. 15OP02) and the PPP Allowance made available by Top Sector Life Sciences & Health to The Dutch Kidney Foundation to stimulate public-private partnerships (grant no. LSHM17034-HSGF).

Conflicts of interest statement

MV received research grants from Vifor, Amgen, Fresenius, and acted as consultant for Medice, Astra Zeneca, Vifor, Amgen, Fresenius, Otsuma, Kyowa Kirin. The other authors declare no conflicts of interest.

Authors contributions

NL, MV and JH conceived the study. NL, MV and CD designed the study. NL wrote the manuscript and MV, JH and CD revised the manuscript. Each author approved the final version of the manuscript.

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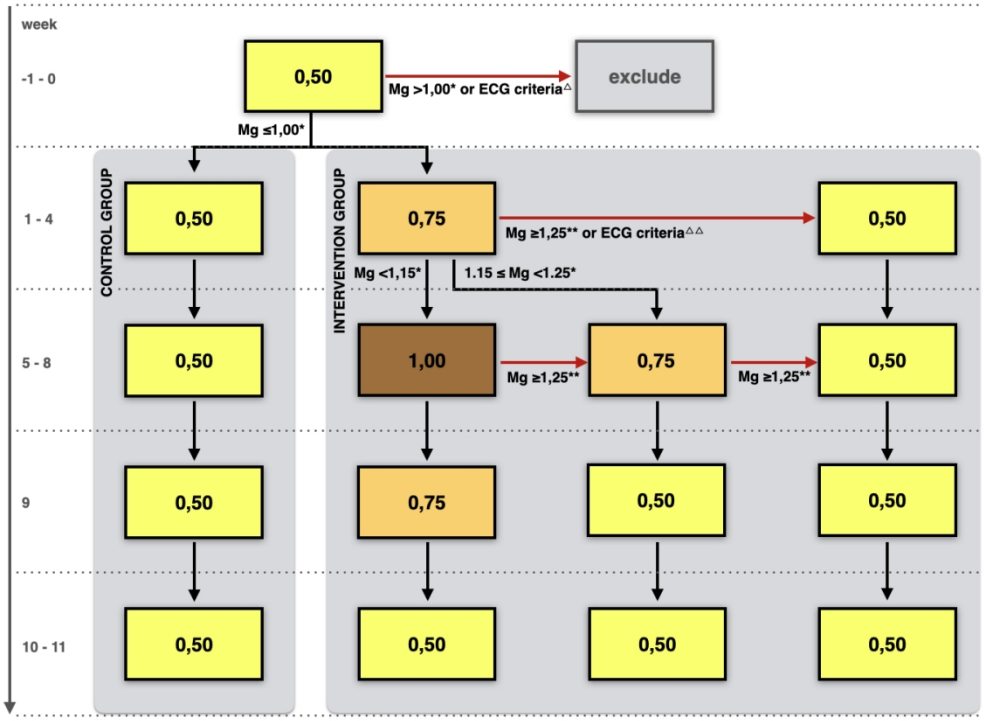
Figure legends

Figure 1. Flow chart of the study intervention. Numbers within frames represent dialysate magnesium concentrations in mmol/L. All other magnesium concentrations represent plasma magnesium concentrations, also in mmol/L. Mg, magnesium; ECG; electrocardiography; $^{\Delta}$, ECG criteria at baseline: bradycardia defined as heartrate <60 bpm or prolonged QTc interval >450ms in male or >460ms in female; $^{\Delta\Delta}$, ECG criteria in week 4: bradycardia with heart rate <50 bpm or prolonged QTc interval >450ms in male or >460ms in female; *, pre-dialysis plasma Mg concentration after the long interdialytic interval; **, plasma Mg concentration at any time point (pre-dialysis and post-dialysis measurements included).

Figure 2. Study procedures. dialysis 1, dialysis 2, dialysis 3: first, second, third dialysis session after the long interdialytic interval of the week respectively; pre: pre-dialysis; post: post-dialysis; blood sampling set 1: lithium heparin gel tube for plasma magnesium measurement; set 2: lithium heparin gel tube for plasma magnesium and potassium measurement; set 3: lithium heparin gel tube for plasma magnesium, potassium, calcium, albumin, phosphate, bicarbonate, CRP, and 2x EDTA tube for hemoglobin and PTH; set A: serum tube for biobanking in participants who provided additional informed consent; set B: 2x EDTA tube for biobanking in participants who provided additional informed consent; ECG: pre-dialysis electrocardiography; Questionnaire: 7-point yes or no questionnaire to ask if a participant experienced the following symptoms in the last week: nausea, vomiting, dizziness, drowsiness, reduced muscle strength, itching and leg cramps, in addition at baseline a question about chronic diarrhea and over-the-counter use of magnesium supplements is included; 3DDD: 3-days dietary diary including 1 dialysis weekday, 1 non-dialysis weekday, 1 non-dialysis weekend day; Holter: 48-hours electrocardiography registration including 1 dialysis session and 1 interdialytic interval; PWV: pulse wave velocity measurement before the mid-week dialysis session.



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483x357mm (72 x 72 DPI)

Week	Dialysate	Blood sampling dialysis 1 pre	dialysis 1 post	dialysis 2 pre	dialysis 2 post	dialysis 3 pre	dialysis 3 post	ECG	Questionnaire	3DDD	Holter	PWV
-1	standard	set 1	set 1	set 1	set 1			X				
0	standard								X	X	X	X
1	study dialysate	set 3+A+B	set 2	set 1	set 1	set 1	set 1					
2	study dialysate	set 1	set 1									
3	study dialysate	set 1	set 1									
4	study dialysate	set 1	set 1					X	X			
5	study dialysate	set 3	set 2	set 1	set 1	set 1	set 1					
6	study dialysate	set 1	set 1									
7	study dialysate	set 1	set 1									
8	study dialysate	set 1	set 1					X	X		X	X
9	study dialysate	set 3+A	set 2									
10	standard	set 1	set 1									
11	standard	set 1	set 1									

Figure 2. Study procedures. dialysis 1, dialysis 2, dialysis 3: first, second, third dialysis session after the long interdialytic interval of the week respectively; pre: pre-dialysis; post: post-dialysis; blood sampling set 1: lithium heparin gel tube for plasma magnesium measurement; set 2: lithium heparin gel tube for plasma magnesium and potassium measurement; set 3: lithium heparin gel tube for plasma magnesium, potassium, calcium, albumin, phosphate, bicarbonate, CRP, and 2x EDTA tube for hemoglobin and PTH; set A: serum tube for biobanking in participants who provided additional informed consent; set B: 2x EDTA tube for biobanking in participants who provided additional informed consent; ECG: pre-dialysis electrocardiography; Questionnaire: 7-point yes or no questionnaire to ask if a participant experienced the following symptoms in the last week: nausea, vomiting, dizziness, drowsiness, reduced muscle strength, itching and leg cramps, in addition at baseline a question about chronic diarrhea and over-the-counter use of magnesium supplements is included; 3DDD: 3-days dietary diary including 1 dialysis weekday, 1 non-dialysis weekday, 1 non-dialysis weekend day; Holter: 48-hours electrocardiography registration including 1 dialysis session and 1 interdialytic interval; PWV: pulse wave velocity measurement before the mid-week dialysis session.

321x127mm (144 x 144 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Title page
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Abstract & 5
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	N/A
Funding	4	Sources and types of financial, material, and other support	15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Title page
	5b	Name and contact information for the trial sponsor	investigator initiated
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
	6b	Explanation for choice of comparators	6-7
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5, 10
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5-6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6-7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8-9

	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	N/A
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9-10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7-8
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10-11
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	5-6
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	10

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	10-11
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7-8, 11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	11
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11
Methods: Monitoring			

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	8-9
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	5
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N/A
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5-7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Figure 2
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	11

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	7-8, Figure 2

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Magnesium in chronic hemodialysis (MAGIC-HD): a study protocol for a randomized controlled trial to determine feasibility and safety of using increased dialysate magnesium concentrations to increase plasma magnesium concentrations in people treated with hemodialysis.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-063524.R2
Article Type:	Protocol
Date Submitted by the Author:	29-Sep-2022
Complete List of Authors:	Leenders, Nicoline; Vrije Universiteit Amsterdam, Department of Nephrology, Amsterdam Cardiovascular Sciences Douma, Caroline; Spaarne Gasthuis Hoofddorp, Department of Nephrology Hoenderop, Joost; Radboudumc, Department of Physiology, Radboud Institute for Molecular Life Sciences Vervloet, Marc; Amsterdam UMC location Vrije Universiteit Amsterdam, Department of Nephrology, Amsterdam Cardiovascular Sciences
Primary Subject Heading:	Renal medicine
Secondary Subject Heading:	Research methods
Keywords:	Dialysis < NEPHROLOGY, NEPHROLOGY, Nephrology < INTERNAL MEDICINE, End stage renal failure < NEPHROLOGY

Magnesium in chronic hemodialysis (MAGIC-HD): a study protocol for a randomized controlled trial to determine feasibility and safety of using increased dialysate magnesium concentrations to increase plasma magnesium concentrations in people treated with hemodialysis.

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Abbreviations

AE, adverse event; CKD, chronic kidney disease; CRP, C-reactive protein; ECG, electrocardiography; PAC, premature atrial complex; PWV, pulse wave velocity; PTH, parathyroid hormone; PVC, premature ventricular complex; SOC, standard of care; SAE, serious adverse event; SPIRIT, Standard Protocol Items: Recommendation for Interventional Trials (SPIRIT)

Abstract

Introduction

People treated with hemodialysis are at increased risk for all-cause and cardiovascular mortality. Plasma magnesium concentration has been inversely associated with these risks. Therefore, plasma magnesium may be a new modifiable risk factor and an increase of dialysate magnesium concentration may be an easy, safe and effective way to increase plasma magnesium concentrations. Detailed information on modulating dialysate magnesium concentrations is limited in literature. Primary objective of this study is to determine the safety and feasibility to increase plasma magnesium concentrations in people treated with hemodialysis by means of sequentially increasing concentration of magnesium in the dialysate.

Methods and analysis

In this randomized double blinded standard of care controlled trial, fifty-three persons treated with hemodialysis, will be randomly allocated 2:1 to either a step-wise individually titrated increase of dialysate magnesium concentration from 0.50 to 0.75 to 1.00 mmol/L during 8 weeks, or a standard dialysate magnesium concentration of 0.50 mmol/L. Other study measurements include dietary records, questionnaires, electrocardiography (ECG), Holter registration and pulse wave velocity. The primary endpoint is pre-dialysis plasma magnesium after the long interdialytic interval at the end of week 8. In addition, the predictive effect of dialysate magnesium concentration and other baseline parameters and dialysis characteristics on plasma magnesium concentration, will be explored using linear mixed models. Safety endpoint is defined by the occurrence of hypermagnesemia above 1.25 mmol/L, or bradycardia or prolonged QTc interval detected on the ECG.

Ethics and dissemination

The study is conducted in accordance with the declaration of Helsinki as revised in 2013 and was approved by the Ethical Committee of the VU University Medical Center. The results of the study will be disseminated by publication in peer-reviewed scientific journals and presentation at national or international conferences in the field of interest.

Trial registration number

NTR 6568 / NL6393

Strengths and limitations

- Blinding and randomization prevents bias occurring from differences in life-style between groups, and enables objective collection and processing of data
- Dialysate concentrations will be individually titrated based on individual plasma magnesium concentrations by an independent physician
- Not only the effects of dialysate magnesium concentrations on plasma magnesium concentrations will be determined, but also the factors that are predictive for these effects
- Major limitation is that the study will not provide information on clinical outcomes including cardiovascular events and mortality, due to limited study duration

Introduction

People with chronic kidney disease (CKD) including those treated with dialysis are at increased risk for all-cause and cardiovascular mortality.[1] This increased risk persists after adjustment for traditional cardiovascular risk factors, indicating that other kidney specific factors contribute to the cardiovascular risk.[1] Recently, lower magnesium has been identified as a potential novel risk factor.[2] Magnesium is involved in many physiological functions, including energy metabolism and regulation of transmembrane transport of ions and consequently, it is essential for muscle function, cardiac rhythm and vascular tone.[3] In a meta-analysis of studies in people with CKD including those on dialysis, we showed that plasma magnesium concentration is inversely associated with all-cause and cardiovascular mortality, and that this not only applies for normal compared to low magnesium, but also revealed protective effects of magnesium above as compared to within the reference range (generally 0.70 – 1.05 mmol/L).[4] Magnesium concentration is also inversely associated with the risk for sudden death and with arrhythmia in people treated with hemodialysis.[5-7] In the general population, serum magnesium has been inversely associated with frequent or complex premature complexes, which predict prognosis including all-cause mortality in the general population.[8, 9] Moreover, serum magnesium was inversely associated with pulse wave velocity (PWV) in people treated with maintenance hemodialysis,[10] and in a short randomized cross-over study in people treated with hemodialysis, higher dialysate magnesium concentration compared to standard dialysate magnesium concentration decreased pulse wave velocity.[11] PWV is a marker of vascular stiffness and a strong predictor of cardiovascular outcome in people with CKD stage 4-5D.[12] In most studies that included multiple categories of plasma magnesium concentration in people with CKD including those treated with dialysis, there was a monotonic inverse association between magnesium and all-cause mortality.[4] We previously showed that a commonly used dialysate magnesium concentration of 0.50 mmol/L in hemodialysis, usually induces a decline of magnesium towards magnesium concentrations in the lower range of normal.[13] Therefore, an increase of dialysate magnesium concentration, may be an easy, safe and effective way to increase plasma magnesium concentrations in people treated with hemodialysis, without the need of oral supplementation. The results of one observational study suggest that there may be an optimal concentration of plasma magnesium in-between 1.15-1.27 mmol/L, with an increasing risk for mortality if magnesium values exceed this range.[14] Although these

findings were not confirmed by other studies that included magnesium values in this high range, this requires to take into account safety when increasing plasma magnesium concentrations.[6, 15, 16] In a previous 4-weeks trial by Bressendorf et al., increasing dialysate magnesium concentration from 0.50 to 1.00 mmol/L resulted in a 0.4 mmol/L increase of plasma magnesium concentration (95%-CI 0.3-0.5).[17] Here, we describe a randomized standard-of-care controlled trial of step-wise increment of dialysate magnesium concentration in people treated with hemodialysis. Primary objective is to determine the feasibility to increase plasma magnesium concentrations in individuals treated with hemodialysis by means of sequentially increasing concentration of magnesium in the dialysate. Secondary objectives are to determine safety of using higher dialysate magnesium concentrations, the effect of dialysate magnesium on plasma magnesium concentration, and to define which parameters are predictive for the increment of plasma magnesium concentration by increasing dialysate magnesium. We will also explore the effects of using higher dialysate magnesium on cardiac rhythm and pulse wave velocity.

Methods

Trial design

In this randomized, double-blind, standard of care (SOC) controlled multi-center trial, individuals treated with hemodialysis will be randomly allocated to either a stepwise increase of dialysate magnesium concentration from 0.50 to 1.00 mmol/L, or continue on a standard dialysate magnesium concentration of 0.50 mmol/L. The protocol was written in accordance with the Standard Protocol Items: Recommendation for Interventional Trials (SPIRIT) and originally prospectively registered at www.trialregister.nl, which is now included in the International Clinical Trial Registry Platform (ICTRP) and can be accessed via https://trialsearch.who.int (registration number NTR 6568 / NL6393).[18] The first participant was randomized in April 2018 and ending of the study is expected at the end of December 2022.

Characteristics of participants and recruitment

Adult persons treated with hemodialysis on a 3-times weekly dialysis schedule, will be enrolled in the study. The in- and exclusion criteria are listed in table 1. Participants will be

recruited from multiple centers in the Netherlands, including Amsterdam University Medical Center location VU Medical Center, Amsterdam; Diapriva dialysis center, Amsterdam; Niercentrum aan de Amstel, Amstelveen; and Spaarne Gasthuis, Hoofddorp. Participants need to provide written informed consent prior to enrollment.

Table 1. Inclusion and exclusion criteria

Inclusion criteria
• Age ≥18 years • Hemodialysis with regular three times weekly dialysis schedule • Hemodialysis since at least 3 months • Standard dialysate magnesium concentration 0.50 mmol/L • Providing informed consent • Pre-dialysis plasma magnesium concentration ≤ 1.00 mmol/L after the long intra-dialytic interval
Exclusion criteria
• Intravenous magnesium supplementation (including total parenteral nutrition) in the last 2 weeks • Expected cessation of dialysis treatment within three months after inclusion or expected permanent or temporary dialysis center switch to a center not participating in the trial within three months after inclusion. • Prolongation of QTc interval: male >450 ms or female >460 ms on baseline ECG • Bradycardia: heart rate below 60 beats per minute on baseline ECG • Chronic arrythmia or cardiac conduction disorder other than atrial fibrillation or ventricular extrasystole that poses the patient at risk at the discretion of the treating physician. • Change of proton pump inhibitor prescription in the last 2 weeks

In order to be eligible to participate in this study, a subject must meet all of the inclusion criteria. A potential subject who meets any of the exclusion criteria will be excluded from participation in this study.

Intervention

In the intervention group, dialysate magnesium is increased stepwise, from 0.50 mmol/L at baseline, to 0.75 mmol/L during week 1-4, and to 1.00 mmol/L during week 5-8. The participant proceeds to the next increment step of dialysate magnesium concentration after week 4 only if pre-dialysis plasma magnesium after the long interdialytic interval is below 1.15 mmol/L in week 4. Otherwise, the dialysate magnesium concentration of 0.75 mmol/L is continued in week 5-8. After week 8, dialysate magnesium will be gradually reduced with 0.25 mmol/L in week 9 and thereafter return to the standard dialysate magnesium concentration of 0.50 mmol/L in week 10.

Participants in the control group are treated with a standard dialysate magnesium concentration of 0.50 mmol/L. (see Figure 1)

The dialysate contains a potassium concentration of 2 or 3 mmol/L, as determined in routine care by the treating physician based on individual needs. For the respective magnesium concentrations, six dialysis concentrates are used in weeks 1-9 (Hemodialysis A-concentrate, D761, D987, D907, D283, D961 and D908, MTN Neubrandenburg GmbH, Neubrandenburg, Germany). In the mineral composition of these concentrates, besides potassium based on individual needs, only the amount of magnesium chloride is different. Calcium concentration is these dialysates 1.25 mmol/L and the acidifier is acetate.

Study procedures and participant time line

The study procedures and participant time line are shown in Figure 2. After informed consent is provided by participants that meet in- and exclusion criteria, blood sampling and electrocardiography (ECG) are performed at baseline. Eligible persons meeting the criteria for plasma magnesium and ECG, as provided in table 1, are allocated to the either SOC or incremental magnesium dialysate. During the trial, blood sampling will be performed before and after the dialysis sessions following the long interdialytic interval weekly, and every dialysis session in week 1 and 5 to measure plasma magnesium concentration. In addition, in week 1, 5 and 9, blood is collected for measurements of potassium, bicarbonate, calcium, albumin, phosphate, parathyroid hormone (PTH), hemoglobin and C-reactive protein (CRP). For laboratory measurements, standard methods of the local laboratory are used. Participants record dietary intake for 3-days at baseline. From this record, dietary magnesium intake is extracted using the Dutch Food Composition Database (NEVO) by using the calculator on the website of the Dutch Nutrition Center.[19, 20] A questionnaire regarding the presence of subjective symptoms that can be associated with hypermagnesemia is completed at baseline, week 4 and 8. This is a 7-point yes or no questionnaire to ask if a participant experienced the following symptoms in the last week: nausea, vomiting, dizziness, drowsiness, reduced muscle strength, itching and leg cramps, and at baseline, also a question about chronic diarrhea and over-the-counter use of magnesium supplements is included. An electrocardiogram (ECG) is repeated before dialysis in week 4 and 8 to determine heart rhythm, frequency and QTc interval. In addition, at baseline and in week 8, participants undergo continuous heart rhythm monitoring and pulse wave velocity (PWV) measurements. Heart rhythm monitoring is performed using a Holter recorder (Fysiologic, Amsterdam, The Netherlands) for 48 hours including one dialysis sessions and one

interdialytic period. Carotid-femoral PWV is measured using the Sphygmocor tonometer (Atcor Medical Pty Ltd, Software version 9.0, Sydney, Australia) preceding the midweek dialysis sessions. Participants are requested to avoid coffee, tea and smoking for 4 hours, and alcohol for 12 hours preceding the measurements, as is recommended by the manufacturer of the device. The patient is placed in supine position, in a quiet environment at room temperature. After attachment of ECG electrodes and bedrest for at least 5 minutes, blood pressure is measured with an automated Omron device at least 3 times with a few minutes in-between, and until no substantial change occurs. Then, the last blood pressure measurement is recorded. The carotid to femoral artery distance is measured directly and multiplied by 0.8 to estimate the difference between cardiac-carotid and cardiac-femoral distance as recommended by expert consensus.[21] The pulse wave is recorded using the tonometer at the carotid and femoral site and then PWV is calculated automatically by the Sphygmocor software from entered blood pressure, distance and ECG and tonometer recordings. Measurements are performed at least twice or until two measurements are of appropriate quality. A measurement is considered of sufficient quality based on the criteria set by the manufacturer: adequate shape of detected signal of ECG and pulse wave, difference of heart rate ≤5 bpm between carotid and femoral measurement, ECG R-tops and pulse wave beginning are correctly identified by the software, and standard deviations of the ECG to carotid and of ECG to femoral time are both <6%. PWV measurement is not performed in participants with irregular heart rhythm, pacemaker rhythm, atrial fibrillation or flutter, heart frequency below 40 or above 160 bpm, 2nd or 3rd degree atrioventricular block, severe aortic valve stenosis or instable carotid plaque, as contra-indicated by the manufacturer. Furthermore, persons' characteristics are recorded at baseline and characteristics of the dialysis are recorded at baseline and weekly for the time of dialysis after the long interdialytic interval and for every dialysis during the first and fifth week of intervention. Recorded dialysis characteristics include modality (hemodialysis or hemodiafiltration), vascular access (catheter, fistula or graft), estimation of dialysis efficiency (Kt/Vurea per session according to Daugirdas' formula), treatment time per session, blood flow, dialysate flow and ultrafiltration volume. Medication use and dosage is recorded at baseline and at week 8. During the trial, all participants receive three times weekly hemodialysis sessions according to their regular schedule. Changes of dialysis schedule during the study will be avoided as much as possible if there is no medical

indication to change the scheme. Also, changes in prescription of proton pump inhibitors and magnesium-containing supplements, laxatives and phosphate binders will be avoided if clinically allowed.

Safety monitoring

If participants in the intervention group reach a plasma magnesium of 1.25 mmol/L or above, as noted by an unblinded independent nephrologist not involved in the trial (see below), at any time point during follow-up in week 1 to 8, dialysate magnesium concentration will be reduced to the previous level in the next week. If plasma magnesium remains 1.25 mmol/L or above in the next week in intervention phase 2 (week 5-8), dialysate magnesium is further reduced one step (to 0.50 mmol/L). If a participant develops bradycardia with heart rate below 50 beats per minute (bpm), or prolonged QTc interval (>450 ms in male or >460 ms in female), noticed on electrocardiography (ECG) in week 4, dialysate magnesium concentration is also reduced to the previous step. (Figure 1) Plasma magnesium above 1.25 mmol/L, bradycardia < 50 bpm and prolonged QTc will be recorded as adverse event (AE). Serious adverse events (SAE's) will be recorded and reported to the primary investigator and medical ethical committee.

Main endpoints

Primary endpoint is pre-dialysis plasma magnesium concentration after the long interdialytic interval at the end of week 8, in the intervention group compared to the control group. The change of pre-dialysis plasma magnesium concentration after the long interdialytic interval from baseline to the end of week 8, will also be determined in the intervention group compared to the control group.

Secondary endpoints

Post-dialysis plasma magnesium concentrations after the dialysis sessions following the long interdialytic interval will also be determined. Safety endpoint is the safety of using higher magnesium concentrations in the dialysate, as indicated by the incidence of respectively hypermagnesemia (>1.25 mmol/L) at any timepoint, or bradycardia (defined as heart rate below 60 bpm) or prolonged duration of QTc interval (>450ms in male or >460ms in female) identified on the ECG in week 4 or 8. Other explorative endpoints include the change of

PWV from baseline to week 8; and the number of complex premature ventricular complexes (complex PVC's), premature atrial complexes (PAC's), and heart rate variability as detected with Holter ECG monitoring. Complex PVC's are defined as PVC's that are multiform, repetitive or have a frequency of >30/h.[8] Furthermore, the following outcomes will be recorded: subjective symptoms that can be associated with hypermagnesemia determined from self-reporting in questionnaires in week 4 and 8; hospitalization; mortality; and cardiovascular events that lead to hospitalization or mortality including arrhythmia, cardiac arrest, acute coronary syndrome, cerebrovascular accident, and hemorrhage from ruptured aneurysms of the abdominal aorta.

Blinding and randomization

Participants that fulfill all screening criteria, will be randomly allocated 2:1 in tranches of 6 to either the intervention group or the control group by the pharmacy according to a computer-generated random list. Participants, treating physicians, nurses and researchers are blinded to treatment allocation. Dialysate cannisters are re-labelled by the pharmacy for the individual participant per individual study week. Labels include information on participant name, date of birth, study week and dialysate potassium concentration, but no further information on dialysate composition. For the first study week, the pharmacy chooses the appropriate dialysate concentrate based on treatment allocation and the in routine individual care determined potassium concentration. From week 2 on, one independent nephrologist that is not blinded for treatment allocation, weekly decides upon the dialysate magnesium concentration, after review of plasma magnesium concentrations according to the algorithm shown in Figure 1. The pharmacy then re-labels the dialysate as prescribed by the independent nephrologist.

Sample size calculation

We previously performed a study to determine plasma magnesium concentrations and variability in people receiving 3-times weekly hemodialysis treatment with a standard dialysate magnesium concentration of 0.50 mmol/L.[13] That study showed a mean pre-dialysis plasma magnesium concentration of 0.88 ± 0.14 mmol/L.[13] After excluding pre-dialysis magnesium levels above 1.00 mmol/L from the analysis, mean pre-dialysis plasma magnesium level was 0.83 mmol/L in that study population. Based on these results, we

expect a mean plasma magnesium concentration of 0.83 ± 0.14 mmol/L in the control group. Based on the results from the CONTRAST cohort analysis, in which plasma magnesium was associated with all-cause and cardiovascular mortality, we consider an increase of plasma magnesium concentration to 0.96 mmol/L in the intervention group relevant, which is a 0.13 mmol/L rise.[5] The required sample size calculated for two independent groups, based on the values just mentioned, a power of 0.80, probability of 0.05, and 2:1 randomization, would be 28 in the intervention and 14 in the control group. To account for an estimated drop-out of 20%, the required sample size is 53 participants in total: 35 in the intervention and 18 in the control group.

Statistical analysis

Continuous variables will be expressed as mean and standard deviation (SD) for normally distributed variables or median and interquartile range (Q1-Q3) for non-parametric distributed variables. Categorical variables will be presented as number and percentage. The primary endpoint, pre-dialysis plasma magnesium after the long interdialytic interval at the end of week 8, will be compared between the intervention and control group, using univariable analysis with an unpaired Student's t-test if variables are normally distributed (if necessary after logarithmic transformation) and with Mann-Whitney U test if variables are non-normally distributed. The analysis is performed as intention to treat, including all participants that are still in study follow-up at week 8. The change of plasma magnesium from baseline to week 8, is first analyzed within each group using a paired Student's t-test, and then the difference from baseline to week 8 (delta) is compared between the groups using linear mixed models. The predictive effect of dialysate magnesium concentration and other baseline parameters and dialysis characteristics on plasma magnesium concentration, will be explored in two separate analyses (for pre-dialysis and post-dialysis concentration) using linear mixed models. In addition, we will explore which parameters are predictive for the increment of plasma magnesium concentration from baseline to week 8 after sequentially increasing dialysate magnesium concentration, using linear mixed models. For secondary endpoints, univariable analysis for within-group changes and between-group differences will be performed using respectively paired and unpaired Student's t-test or Mann-Whitney U tests for continuous variables, and chi-quadrate or Fisher's exact test for

dichotomous variables. Linear mixed models will be used for multivariable analysis of secondary endpoints with repeated measures.

Data management

Data is recorded in electronic case report forms using the webbased software Castor EDC (Amsterdam, The Netherlands). All data is stored in coded form. The identification key is stored at the local study site only. Randomization codes are stored at the pharmacy. The randomization code will not be broken until follow-up of all participants is completed.

Ethics and dissemination

The study is conducted in accordance with the declaration of Helsinki as revised in 2013 and was approved by the Ethical Committee of the VU University Medical Center (registration number 2017.408, NL62679.029.17).

The results of this study will be offered for publication in international peer-reviewed journals. In addition, the results can be presented at national and international conferences and meetings in the field.

Patient and public involvement

This study protocol was reviewed and approved by the Dutch Kidney Foundation (DKF). A patient panel of the Dutch Renal Patients Association is involved in the review of research protocols submitted to the DKF. Investigators will communicate results to participants once the final results become available. The results will also be shared with patient organizations.

Discussion

This study aims to determine feasibility, safety, and predictive parameters for the effect of using dialysate with higher magnesium concentration to increase plasma magnesium concentrations in people treated with hemodialysis. In addition, this study will explore effects of using higher dialysate magnesium on cardiac rhythm and pulse wave velocity.

Relation with previous studies

In a previous study, we demonstrated that a commonly used dialysate magnesium concentration of 0.50 mmol/L generally induces a decline of plasma magnesium concentrations towards concentrations in the lower range of normal.[13] Detailed information in literature on the effects of increasing dialysate magnesium concentration on pre- and post-dialysis plasma magnesium concentrations and safety is sparse. Two other studies showed that a dialysate magnesium concentration of 0.75 mmol/L generally resulted in a relatively stable plasma magnesium concentration, with a mean pre-dialysis concentration of 1.2 mmol/L and mean post-dialysis concentrations of 1.1 up to 1.2 mmol/L.[22, 23] In another trial, a 4-weeks single-step increment of dialysate magnesium concentration from 0.50 to 1.00 mmol/L resulted in a 0.4 mmol/L increase (95%-CI 0.3-0.5) of pre-dialysis plasma magnesium concentration and a mean pre-dialysis plasma Mg concentration of 1.4 ± 0.2 mmol/L .[17] That study did not perform ECG nor Holter monitoring. As outlined in the introduction, an observational study in people treated with hemodialysis found an inverse association between plasma magnesium concentrations and arrhythmia.[7] Moreover, in a short randomized cross-over study in people treated with hemodialysis, a dialysate magnesium concentration of 0.75 mmol/L compared to 0.50 mmol/L decreased pulse wave velocity.[11]

Strengths and limitations

The strengths of this study protocol are the blinding and randomization. Although the primary outcome is an objective outcome measure, blinding and randomization are essential to prevent bias occurring from changes in life styles including dietary magnesium intake. In addition, it is of relevance for objective collection and processing of data from questionnaires, pulse wave velocity measurements, electrocardiography, and reporting of SAE's. Another strength of this protocol is that dialysate magnesium concentration will be individually titrated based on individual plasma magnesium concentrations. In addition, not only the effect of dialysate magnesium will be determined, but also other factors that are predictive for this effect will be determined. The major limitation of this protocol, is that the study will not provide information on clinical outcomes including cardiovascular events and mortality, due to a limited duration of the study.

Potential impact

The study described in this protocol may provide relevant information on the effect of dialysate magnesium on plasma magnesium concentrations, the strategy for titration of dialysate magnesium based on individual needs, the safety of increasing plasma magnesium concentrations, and about factors that are predictive for the effect of dialysate magnesium on plasma magnesium concentration. This information may enable to safely increase plasma magnesium concentrations by individualized dialysate magnesium concentrations. In addition, this study will provide explorative data about the effects of increased dialysate magnesium concentration on intermediate cardiovascular outcomes including cardiac rhythm and PWV. The information provided by this study, may pave the way to larger long-term randomized controlled trials on the effects of increasing plasma magnesium concentrations on clinical outcomes including all-cause and cardiovascular mortality in people treated with hemodialysis. If plasma magnesium indeed improves clinically relevant outcomes, and can be safely increased by means of individualized increasing dialysate magnesium concentrations, potentially large health benefits may be achieved if magnesium is increased slightly above the reference range by an increase of dialysate magnesium concentration. If so, the cost-effect ratio is likely low, as raising magnesium concentration is an inexpensive intervention. In addition, it would be an easy intervention that needs no additional patient effort and no oral supplementation would be needed in these persons that already experience a high pill burden. Therefore, the study described in this protocol may provide information of high relevance to patients, clinicians and health care providers and may eventually help to decrease morbidity and mortality in people treated with hemodialysis.

Safety considerations

Plasma magnesium concentrations are expected to rise up to values above the reference range by the increase of dialysate magnesium concentration. However, clinical symptoms of hypermagnesemia, typically are not observed before plasma magnesium concentrations exceed 2.0 mmol/L, which is high above the target concentrations in this study. Reported symptoms of hypermagnesemia (if plasma concentrations are above 2.0 mmol/L) include lethargy, drowsiness, flushing, nausea and vomiting , and diminished deep tendon reflexes.[24] In even more severe hypermagnesemia (plasma concentrations above 3.0 mmol/L) also somnolence, loss of deep tendon reflexes, hypotension and ECG changes can

occur, and in extreme hypermagnesemia (above 5.0 mmol/L) complete heart block, cardiac arrest, apnea, paralysis and coma have been reported[24]. As a result of hemodialysis inherent techniques, the increment of (free) plasma magnesium is restricted by its concentration in the dialysate (maximally 1.00 mmol/L in this study), so overshoot to symptomatic concentrations is virtually impossible. The risk for severe or symptomatic hypermagnesemia is further minimized by intensive monitoring. As dialysate magnesium concentration is not further increased after a plasma magnesium concentration of 1.15 mmol/L is reached, and is reduced if plasma concentrations of 1.25 mmol/L are reached at any time point, severe or symptomatic hypermagnesemia will be prevented. These maximal target concentrations are set taking into account an observational study in people treated with hemodialysis that suggested an optimal magnesium concentration in-between 1.15-1.27 mmol/L and increased risk for mortality if magnesium exceeds 1.27 mmol/L.[14] Furthermore, for safety reasons, individuals with bradycardia or a prolonged QTc interval on the ECG at baseline will be excluded from participation, and in individuals with bradycardia with a heart rate below 50 bpm or a prolonged QTc interval identified on the ECG in week 4, dialysate magnesium will be reduced. Based on these careful methods, the risk for individuals participating in this study is low. Considering the limited burden and risks associated with this study and a possible highly-relevant contribution to future improvement of treatment and prognosis in people treated with hemodialysis, the potential benefits outweigh the burden and possible risks.

Trial status

The trial is currently ongoing. The first participant was randomized on the 4th of April 2018 and up till now 43 of 53 participants have been randomized.

Funding

This work was supported by the Dutch Kidney Foundation (PhD grant no. 15OP02) and the PPP Allowance made available by Top Sector Life Sciences & Health to The Dutch Kidney Foundation to stimulate public-private partnerships (grant no. LSHM17034-HSGF).

Conflicts of interest statement

MV received research grants from Vifor, Amgen, Fresenius, and acted as consultant for Medice, Astra Zeneca, Vifor, Amgen, Fresenius, Otsuma, Kyowa Kirin. The other authors declare no conflicts of interest.

Authors contributions

NL, MV and JH conceived the study. NL, MV and CD designed the study. NL wrote the manuscript and MV, JH and CD revised the manuscript. Each author approved the final version of the manuscript.

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Figure legends

Figure 1. Flow chart of the study intervention. Numbers within frames represent dialysate magnesium concentrations in mmol/L. All other magnesium concentrations represent plasma magnesium concentrations, also in mmol/L. Mg, magnesium; ECG; electrocardiography; $^{\Delta}$, ECG criteria at baseline: bradycardia defined as heartrate <60 bpm or prolonged QTc interval >450ms in male or >460ms in female; $^{\Delta\Delta}$, ECG criteria in week 4: bradycardia with heart rate <50 bpm or prolonged QTc interval >450ms in male or >460ms in female; *, pre-dialysis plasma Mg concentration after the long interdialytic interval; **, plasma Mg concentration at any time point (pre-dialysis and post-dialysis measurements included).

Figure 2. Study procedures. dialysis 1, dialysis 2, dialysis 3: first, second, third dialysis session after the long interdialytic interval of the week respectively; pre: pre-dialysis; post: post-dialysis; blood sampling set 1: lithium heparin gel tube for plasma magnesium measurement; set 2: lithium heparin gel tube for plasma magnesium and potassium measurement; set 3: lithium heparin gel tube for plasma magnesium, potassium, calcium, albumin, phosphate, bicarbonate, CRP, and 2x EDTA tube for hemoglobin and PTH; set A: serum tube for biobanking in participants who provided additional informed consent; set B: 2x EDTA tube for biobanking in participants who provided additional informed consent; ECG: pre-dialysis electrocardiography; Questionnaire: 7-point yes or no questionnaire to ask if a participant experienced the following symptoms in the last week: nausea, vomiting, dizziness, drowsiness, reduced muscle strength, itching and leg cramps, in addition at baseline a question about chronic diarrhea and over-the-counter use of magnesium supplements is included; 3DDD: 3-days dietary diary including 1 dialysis weekday, 1 non-dialysis weekday, 1 non-dialysis weekend day; Holter: 48-hours electrocardiography registration including 1 dialysis session and 1 interdialytic interval; PWV: pulse wave velocity measurement before the mid-week dialysis session.

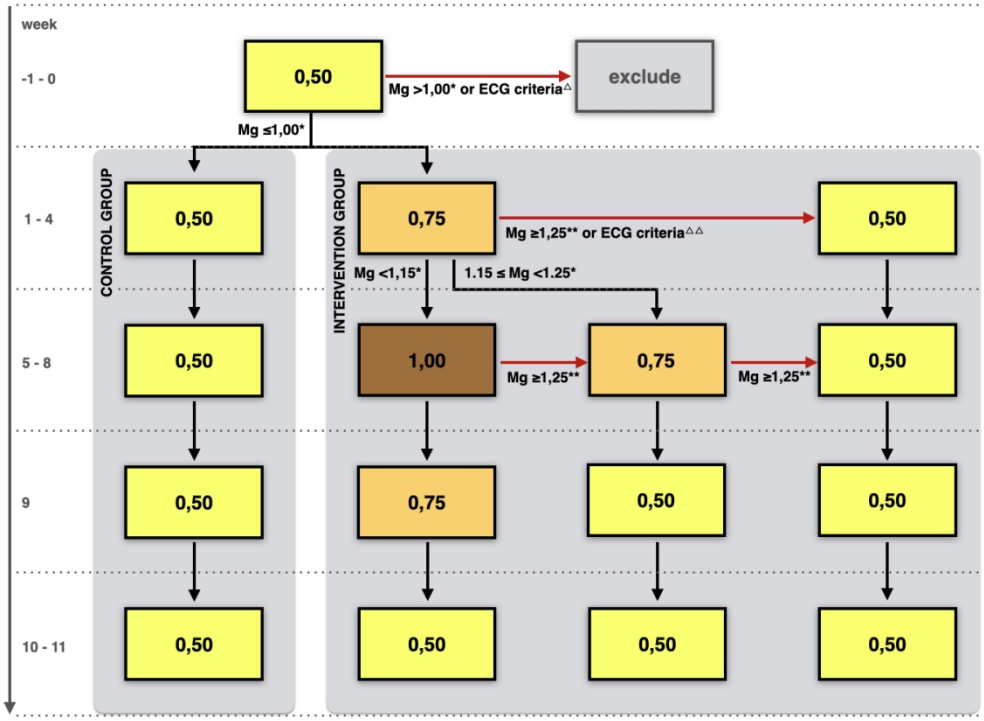

Figure 1. Flow chart of the study intervention. Numbers within frames represent dialysate magnesium concentrations in mmol/L. All other magnesium concentrations represent plasma magnesium concentrations, also in mmol/L. Mg, magnesium; ECG; electrocardiography; △, ECG criteria at baseline: bradycardia defined as heartrate <60 bpm or prolonged QTc interval >450ms in male or >460ms in female; △△, ECG criteria in week 4: bradycardia with heart rate <50 bpm or prolonged QTc interval >450ms in male or >460ms in female; *, pre-dialysis plasma Mg concentration after the long interdialytic interval; **, plasma Mg concentration at any time point (pre-dialysis and post-dialysis measurements included).

483x357mm (72 x 72 DPI)

Week	Dialysate	Blood sampling dialysis 1 pre	dialysis 1 post	dialysis 2 pre	dialysis 2 post	dialysis 3 pre	dialysis 3 post	ECG	Questionnaire	3DDD	Holter	PWV
-1	standard	set 1	set 1	set 1	set 1			X				
0	standard								X	X	X	X
1	study dialysate	set 3+A+B	set 2	set 1	set 1	set 1	set 1					
2	study dialysate	set 1	set 1									
3	study dialysate	set 1	set 1									
4	study dialysate	set 1	set 1					X	X			
5	study dialysate	set 3	set 2	set 1	set 1	set 1	set 1					
6	study dialysate	set 1	set 1									
7	study dialysate	set 1	set 1									
8	study dialysate	set 1	set 1					X	X		X	X
9	study dialysate	set 3+A	set 2									
10	standard	set 1	set 1									
11	standard	set 1	set 1									

Figure 2. Study procedures. dialysis 1, dialysis 2, dialysis 3: first, second, third dialysis session after the long interdialytic interval of the week respectively; pre: pre-dialysis; post: post-dialysis; blood sampling set 1: lithium heparin gel tube for plasma magnesium measurement; set 2: lithium heparin gel tube for plasma magnesium and potassium measurement; set 3: lithium heparin gel tube for plasma magnesium, potassium, calcium, albumin, phosphate, bicarbonate, CRP, and 2x EDTA tube for hemoglobin and PTH; set A: serum tube for biobanking in participants who provided additional informed consent; set B: 2x EDTA tube for biobanking in participants who provided additional informed consent; ECG: pre-dialysis electrocardiography; Questionnaire: 7-point yes or no questionnaire to ask if a participant experienced the following symptoms in the last week: nausea, vomiting, dizziness, drowsiness, reduced muscle strength, itching and leg cramps, in addition at baseline a question about chronic diarrhea and over-the-counter use of magnesium supplements is included; 3DDD: 3-days dietary diary including 1 dialysis weekday, 1 non-dialysis weekday, 1 non-dialysis weekend day; Holter: 48-hours electrocardiography registration including 1 dialysis session and 1 interdialytic interval; PWV: pulse wave velocity measurement before the mid-week dialysis session.

321x127mm (144 x 144 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Title page
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Abstract & 5
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	N/A
Funding	4	Sources and types of financial, material, and other support	15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Title page
	5b	Name and contact information for the trial sponsor	investigator initiated
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
	6b	Explanation for choice of comparators	6-7
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5, 10
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5-6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6-7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8-9

	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	N/A
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9-10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7-8
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10-11
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	5-6
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	10

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	10-11
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7-8, 11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	11
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11
Methods: Monitoring			

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	8-9
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	5
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N/A
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5-7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Figure 2
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	11

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	supp
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	7-8, Figure 2

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.